CHEMICAL RISK ASSESSMENT is *Literally* a Scientific Fraud

..And Has Contributed to A ***"Nightmare Carved In Molasses"***[1]

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BY TONY TWEEDALE
Missoula, MT
ttweed@wildrockies.org
tel.: 406-721-5420

Abstract: **Quantitative risk assessment (Q-RA) as performed on environmental agents such as toxic chemicals, far from being a "science" as its proponents often claim it to be, is literally a scientific fraud**. After briefly reviewing how chemical Q-RA is performed, I exhaustively prove the eight ways that it is a scientific fraud. Next I summarize the situation in monitoring human exposure, followed by an assesment of the tragic massive dearth of data on time trends in human chronic diseases, but this nevertheless shows that the incidence of many chronic diseases has (and seems to have) exploded in this era of omnipresent, intimate chemical exposures. Finally, to definitively prove that chemical Q-RA fails to protect the biosphere from being poisoned, I exhaustively compare the toxicology data generated by Q-RA methods with the data from the independent literature (i.e. free from financial conflicts), for pesticides--one of the only two categories of chemicals which in theory undergo pre-market testing for safety!

FREQUENTLY USED ACRONYMS

a.i.	active ingredient in pesticide fornulations
ADI	Acceptable Daily Intake (equivalent to an RfD)
b.w.	body weight
BDNF	Beaverhead-Deerlodge National Forest
BMD	Benchmark Dose
BNF	Bitterroot National Forest
BP-A	bisPhenol-A
BPA	Bonneville Power Administration
CBI	Confidential Business Information
CoI	Conflict of interests
D/R	Dose/Response relation
DQA	Data Quality Act
EA	Environmental Assessment (precedes a DEIS)
EIS (DEIS, FEIS)	Environmental Impact Statement (Draft or Final)
EPA	U.S. Environmental Protection Agency
FIFRA	Federal Insecticide, Fungicide & Rodenticide Act
FoIA	Freedom of Information Act
FONSI	Finding of No Significant Impact (necessary to avoid an EIS)
HCB	Hexachlorobenzene
LD-50	Lethal Dose-50% (the acute dose that kills 50% of test organisms)
LNF	Lolo National Forest
L/NOEL, L/NOAEL	Lowest or No Observable (Adverse) Effect Level--*all are equivalent*
MCPA	MonoChloro Phenoxy Acetic Acid
MoS	Margin of Safety
MTD	Maximally Tolerated Dose
NTP	National Toxicology Program (the federal interagency testing program)
Q-RA	Quantitative Risk Assessment
RED	re-Registration Eligibility Doc. (rationale of a pesticide's registration)
RfD	Reference Dose (estimated safe dose for non-cancer effects)
TSCA	Toxic Substances Control Act
USFS	U.S. Forest Service

HOW IS QUANTITATIVE RISK ASSESSMENT for CHEMICALS PERFORMED?[2]

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[1] The author of this aphorism is unknown to me, but I would love to know, so contact me if you know it.

[2] C. Klaasen, ed. 'Casarett & Doull's Toxicology: the basic science of poisons' 6th. ed. New York City: McGraw Hill 2001; **and** 'Sax's Dangerous Properties of Industrial Materials' (3 vol.'s); are classic texts of traditional toxicology, that verify the facts presented in this sub-section, a primer on Q-RA.

All complete chemical quantitative risk assessments (Q-RAs) use a common methodology of three steps: hazard analysis, exposure analysis, and the resulting risk analysis. First the chemical's known hazards and the potency of the hazard are reviewed in a literature search; second, a delivered dose from many exposure scenarios is estimated; finally, the risk analysis simply compares the estimated exposure's dose to the first step's putatively safe dose, to see if it is exceeded; if so, by how much. The amount of number-crunching in the exposure analysis step is considerable because each of the many exposure pathways--from release to the delivered dose to the receptor tissue--has many steps at which the modeled concentration of the chemical may change. That number-crunching is facilitated by using computer models that integrate the final two, even all three stages of chemical Q-RA.

Chemical Q-RA uses both human and animal toxicity data, but because most societies find it unethical to experiment on humans, the most specific information used in chemical Q-RA (especially on the dose-response relation) comes from animal experiments--where most of the otherwise-uncontrolable variables of the human experience (which may affect the variable studied, i.e. the toxic effect of a chemical) can be controlled. Human responses are obviously more relevant, so when available (e.g. if exposure levels at work are measured), are used in chemical Q-RA.

Chemical Q-RA purports to assess all risks of a chemical, acute and chronic. Acute effects are those caused immediately (roughly, within weeks or months, but usually much quicker), so typically they require a high, often single, dose. They are typically very evident and serious effects, though an organism may recover fully--in short an acute effect is a poisoning. Accordingly, acute toxicity test protocols use high doses and look for rapid and obvious health effects. Acute exposures are relatively rare--examples involving pesticides include: aquatic life effects after a spill, a person entering a pesticide application area too soon, or an inappropriate use by a pesticide applicator. The dominant acute test protocol is the 'Lethal Dose-50%' (LD-50)--the dose that kills half the test organisms, typically within four days (96 hr). Acute effects other than death are not often tested in chemical Q-RA/toxicology. If the dose delivered to the organism is unknown, the test protocol is slightly modified to measure the effect of that concentration in the organism's surrounding air or water, and renamed the Lethal Concentration-50% (LC-50) test.

By definition, chronic effects (and doses) are all non-acute ones, i.e. repeated dosing over a period that eventually may elicit an injury. As with acute toxicity, the nature of chronic toxicity drives the basic protocol for testing for it: regular doses, lower than in acute toxicity tests, are given over longer periods--ideally two-thirds of the animals' lifetime--to approximate the risk of a lifetime of chronic exposure.

Acute exposure toxicity tests, though designed to test a different risk (poisoning, i.e. acute illness) than chronic disease, are fundamentally of less value than chronic toxicity tests. First, obviously far more exposures occur at lower levels of toxins than do at higher levels--low dose exposures are ubiquitous (at some level), both intuitively and as verified by billions of chemical analysis of our environment. Second, chronic effects may be as severe as acute poisonings, yet are lalso ikely to be less reversible (such as cancer, birth defects). **Thus, chronic exposure toxicity is the natural focus of toxicology and of chemical Q-RA**.

All chronic toxicity tests *evaluate cancer risk more conservatively than non-cancer risks, due to* the biologically-plausible possibility that cancerous tumors may grow from a single gene mutation in a single cell--a long-accepted theory, for which there is now persuasive evidence.[3] The chemical's cancer potency (also called the 'cancer slope factor') in a group of test animals is estimated by a total reliance on the assumption that 'the dose makes the poison'--that higher doses cause ever worse effects. Non-cancer chronic tests rely on this concept too; which will shortly be critiqued.

So in carcinogenicity tests, the different number of cancerous tumors that different high doses (two or three) of a chemical may elicit are extrapolated (so long as more cancers resulted at each higher dose) linearly down to the dose that would result in as many cancers in the population as society accepts (typically, one-in-a-million).[4] By assuming that the dose/response (D/R) relation is positive and linear at all doses, the cancer potency at the high test doses may be extrapolated to any dose. But for uniformity in comparing the cancer potency of various chemicals, the potency is typically stated at just one dose:[5] 1 mg/kg of body weight (b.w.) per day. Usually that potency will be a fraction of a cancer; for some potent carcinogens, greater than one cancerous tumor. That strange metric is due to expressing the observed number of tumors in a group of test animals as if it applied to a single animal (including human). Expressing the cancer potency at the '1 mg/kg of b.w. per day' dose also conveniently uses the same unit that non-cancer chronic tests use.

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[3] Nancy Segal 1 Aug. 2003 'X and Y, A Wild Pair' Science:301:599 (book review, see 5th p'graph).

[4] This becomes the basis for a chemical's regulation: back-calculate from the acceptable dose the allowable levels in the environment, thence to the allowable emissions from sources (for non-cancer risk regulation too).

[5] A mass dose is usually expressed over time, and often expressed as the resulting concentration, e.g.'mg/kg of b.w. per day'. Also, note that concentrations in different media cannot be expressed as a simple proportion (e.g. ppb) due to different densities--e.g. the shifting density of the fixed number and mass of molecules in a volume of air.

Non-cancer chronic risk tests are less conservative than cancer tests due to their use of another assumption, the 'threshold dose'--that no adverse effect can possibly occur below the No or Lowest Observable Adverse Effect Level (NOAEL or LOAEL, sometimes called just NOEL or LOEL). Typically shown on a simple X-Y graph, the *cancer* dose-response curve is linear down to zero; but the *non-cancer* "threshold dose" curve is hockey-stick shaped, making visible the assumption that no effect can occur below the dose at which the curve reads 'zero effect'.

Several methodologies are used to set a safe level for non-cancer effects, but all are similar to dominant one, US-EPA's Reference Dose (RfD) metric. They divide the (assumed) threshold dose, the "NOEL" by an arbitrary safety factor. The safety factor is usually 100 fold: 10-fold to supposedly account for inter-species variation in response, and 10-fold to supposedly account for intra-species (i.e. individual) variation. A third safety factor of up to 10-fold more may also be factored in; depending on failures of the quality, robustness and sufficiency of the experiment's protocols and its resulting data.

If non-cancer chronic tests thoroughly tested the whole spectrum of doses and at realistically short intervals--to be sure what the NOAEL is--then non-cancer chronic toxicity tests might be more conservative than cancer toxicity tests; however, non-cancer tests also just test at near the highest tolerable dose.

WHY IS CHEMICAL Q-RA a SCIENTIFC FRAUD?

"Science" means to test a hypopthesis, i.e. to produce results that can be objectively replicated (or not) by outsiders, including critics (coincidentally, that's why full transparency is indispensable in science--"publish or die"). **We demonstrate below that chemical Q-RA is *not* science, because it fails even to test the hypothesis that it claims to prove: that certain exposure levels to chemicals are safe; rather it is a scientific fraud.**

Perhaps humans' evolution in a challenging environment promoted an instinct that problems be called solved, even if the solution is not overall beneficial to human society. This would help explain how the scientific fraud of chemical Q-RA swiftly became the basis of all regulation of chemical (and other technologies) risks in the United States; including many of the risks are evaluated in Environmental Impact Statements, EIS.

Chemical Q-RA is almost exclusively relied on by chemical risk managers (agencies, policy makers, etc.) to assign an allowable ("safe") exposure to chemicals. For instance, in the USA the permission to sell and use pesticides--the only non-therapeutic (e.g. chemotherapy) chemicals in commerce *intended* to be poisons--is based on Q-RA. Q-RA is possibly the most developed of a risk manager's broader tool-kit of cost-benefit analysis (C/BA) tools. C/BA has many of the same deadly flaws that Q-RA has, but critiquing it is beyond the scope of this paper.

Chemical Q-RA's authoritative aura--the result of its output of numericized (precise-appearing) answers to what are in fact complex and data-impoverished questions about risk[6]--is extraordinarily dangerous.[7] It masks assumptions, complexities and data-gaps, as follows:[8]

- Just a few of many possible health effects are evaluated before "no-risk" is proclaimed;
- Tests begin at late adolescence, missing the exquisitely vulnerable time of development;[9]
- The "safety factors" on the lowest adverse dose, to lessen non-cancer risk, are entirely random;
- The estimated risk is for one chemical--that ignores any synergistic effects of the chemical brew that organisms

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[6] Risk is roughly: (probability of an occurrence) x (consequence). Thus chemical risk is roughly 'exposure x toxicity'.

[7] "Risk assessment data can be like a captured spy. Torture it enough, it'll tell you anything you want to know."
-W. Ruckleshaus 1984 (then Admin. of EPA) in 'Risk in a Free Society' Risk Analysis:4:3:157-62.
"Risk assessment is like searching for your lost keys only under the circle of light of the streetlamp." -?

[8] That these problems are present in the fundamental protocols of chronic effects tests can be verified in C. Klaasen (ed.) 2000 **or** D. Rall 1991 'Carcinogenesis & Human Health: part 2' Science:252:10-11 (letters) **or** EPA/Office of Prevention, Pesticides & Toxic Substances (OPPTS) Aug. 1998 'Health Effects Test Guidelines: OPPTS 870.4100 Chronic Toxicity', EPA-712-C-98-210. This (and similar ones for cancer & developmental (in utero) tests)EPA Guidances are used by the U.S. and OECD (Organization for Economic & Cooperative Development) governments to attempt to harmonize safety tests regulations for pesticides and other chemicals.

[9] Except in developmental (in-utero) tests, and a few new test protocols.

throughout the biosphere are actually exposed to;

- The tests rely entirely on the often-false assumption that greater doses cause more disease, therefore, a simplistic-linear dose/response relation is assumed at all doses tested; *unless*...

- ...*it is being assumed that there can be no effect below a given dose*. Other than for cancer, it is assumed that no effect can occur below unrealistically high doses. The much lower actual exposures in the real-world exposures are assumed to be entirely safe if a cursory look for a few toxic effects at those very high test doses finds no effects. Even cancer tests, which don't make this assumption, do not actually test realistic doses--instead they linearly extrapolate the cancer potency from the high doses tested down to zero dose, to estimate the risk at all doses.

This system of risk management for chemicals endures even as science slowly gathers the evidence that our species, at least, is suffering increasing rates of diseases not otherwise explainable (see the following major section). It endures even as the instruments of biochemistry for several decades, at an accelerating pace, are showing toxicity from environmental agents at our ubiquitous low real-world doses.

PROBLEM 1: THE DOSE DOES *NOT* MAKE THE POISON! This is chemical Q-RA's most obvious scientific fraud: not to test the effect of the actual doses of daily life, which are cumulative and low-dose (called "environmentally relevant" doses). To perpetrate this crucial deceit, Q-RA and classic toxicology must rely on a one key assumption: that "the dose makes the poison".

This is actually a dual assumption: that both a positive, and a linear, dose/response (D/R) relation exists--i.e. that increasing doses of an agent cause ever more injury.

This fraud developed due to expense, as follows. Chronic diseases are typically rare enough in populations that accurately testing a chemical for such a disease on animals would seem to require huge populations of test animals...*unless* "the dose makes the poison" assumption can be employed!. Due to the relative rarity of many chronic diseases, low test doses may not elicit any case of a chronic disease in a population of a few hundred test animals. But, if the chemical causes the disease, at very high doses--close to as much as can be acutely tolerated--its potency should detactable. Using this response rate at very high test doses, the incidence rate of the disease at the much lower real-world doses is predicted with a simple linear extrapolation from the test doses (and relying on the linear D/R relation assumption). In sum, depending on a chemical's potency, it is assumed that thousands to tens of thousands of test animals would be needed to detect these relatively rare diseases; if actually-experienced doses were tested for their toxicity. Because the proper care, dosing and pathological examination of test animals is expensive, this dual, untested assumption--that higher doses cause ever more disease, linearly--allows a bearable expense per test, often well below $1 million per chemical tested.

In the typical chronic toxicity test protocol (still universally used), two to eight hundred animals (two mammalian species of both sexes, grouped into two or three dose levels, plus a negative control group at zero dose) are assumed to be enough to reliably estimate the toxic potency of a chemical (statistically, the 95% confidence level is usually used, i.e. the likelyhood that the same result would be obtained in 95 out of 100 repetions of the experiment).

The universal chronic toxcity protocol proceeds as follows. First a "semi-chronic exposure" test is performed, to find its Maximally Tolerated Dose (MTD). Starting with the chemical's acute lethality dose (LD-50, the dose that kills 50% of the test animals), the MTD is the dose that is just short of death or immobility that over a medium period--typically 90 days--begins to approximate the longer period of a chronic toxicity test (theoretically, at least two-thirds the animal's life), but which doesn't take nearly as long to establish. The MTD then becomes the 'high' dose in the actual chronic effect test. Typically the 'mid' and 'low' dose levels--at half and 1/10th or (rarely at 1/100th or 1/1,000th) the MTD--are the only other doses selected; sometimes just a high (MTD) and a low (perhaps 1/2 the MTD) dose--this regime is the bare minimum needed to establish a linear (presumably) D/R curve and then extrapolate, employing the crucial assumptions discussed. Even the uncommom one-hundredth the MTD is almost always far higher than real-world exposure to a chemical. A 1/1,000th MTD sometimes overlaps real-world exposure levels, but it is an extremelly rare test level, as toxicologists fear that low dose may not elicit a detectable incidence of what are relatively rare chronic diseases in their relatively small group of test animals. That's why they almost invariably test at 1/2 to 1/100th the MTD.

We next show how wrong this crucial "dose makes the poison" assumption is.[10]

We must first travel to the organic (i.e. carbon-based) chemistry revolution, responsible for the explosion in the number of dangerous chemicals in commerce. It originated in the 1820's when German and French chemists began to investigate the atomic composition of molecules, and began to substitute other atoms onto these carbon backbones (including--even then!--the toxin chlorine). But it was classic German thoroughness that enabled this research to be commercialized ; i.e. only the Germans invested in the science and engineering infrastructure necessary to carry out the tens of thousands of tests on the almost endless number of organic compounds needed to discover and develop a single organic chemical product;[11] the synthetic dye industry was the first to profit from this systematic procedure, by about 1855. It spawned a commercial revolution, the age of chemistry (roughly, the 20th Century), which not incidentaly featured the planet's first effective worldwide cartels: oligopolies and geographic monopoly arrangements; relying on intellectual property laws, which in turn relied and thrived on the commercialization of organic chemistry.[12] Inorganic chemistry (such as toxic metals) also continued to grow commercially, but it was swamped by the revenues and the sheer variety of compounds from organic chemistry; justifing the tag, 'the organic chemistry revolution'.

An ancillary to this intellectual/commercial revolution was the development of toxicology as a professional science, it coalesced by the 1920s. **The organizing paradigm of this field**, even today, is captured by that cliché originated by 15th-century Swiss physician and alchemist Paracelsus: 'The dose make the poison'[13] That this cliché is today still the organizing paradigm of traditional toxicology is proved simply by its primary and necessary place in toxicity testing, as described above. Classic toxicology and its organizing maxim are the key to understanding the calamitous social failure that chemical Q-RA is.

Paracelsus' maxim is from the Middle Ages, when science had no methods to associate most ill-effects with a possible cause; unless the putative causative agent was immediate in time or place to the disease or poisoning, thus causing an immediate and obvious effect. To Paracelsus 500 years ago, the strong correlation of poison doses with acute diseases must have been obvious, once he began examining the evidence. In poisoning and most other acute disease, a positive dose/response relation is often evident. *Today, the very fact that we try to test for chronic (long-term) exposure effects proves that we know this is **not** a general rule*.

Certainly, it is a very common occurance that 'the dose makes the poison'--ionizing radiation's damage to DNA is one obvoius and ubiquitous example. That diseases often occur at the area most highly exposed (e.g. the famous first association in the late18th Century of cancer with an occupation--Dr. Percival Pott's chimney-sweepers) implies that the dose makes the poison. Obviously--in many instances--there is a "threshold dose" below which a disease does not occur. In short, the dose often does make the poison.

But science proceeds as its tools improve, and recent decades have seen the overturning of this famous paradigm, as we learned how to see the effects of environmentally-relevant doses. In thousands of observed instances (and therefore certainly in millions of other situations), there is no linear D/R response relation; i.e. the dose does *not* make the poison. **After all, one defining characteristic of life is its nuance**.

One critical variable is, 'timing--not the dose--makes the poison'[14] especially during development (for more, see Problem #3, below). **Or**, often lesser exposures will cause greater injury--see the following discussion for many examples and mechanisms.

For example: DNA microarrays enable hundreds of tests of functioning DNA to be performed at once--a recent paper (which actually published much of such voluminous raw data) shows that about 15% of several hundred genes responding (to produce or suppress the protein produced by the gene) to two dose levels of hexachlorobenzene (a

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[10] In addition, a recent report by an highly qualified activist group devotes a major portion to explain the dangerous fallacies of high-dose toxicity tests, **giving many additional examples of results contrary to the assumption, and a couple of exceptional graphics to illustrate the point**: Environmental Working Group (EWG) 2003 'Body Burden: the pollution in people' Wash. D.C. Available at http://www.ewg.org/reports/bodyburden -- see the two sections begining with 'Can low doses of chemicals hurt me?'.

[11] Ursula Klein 5 Nov. 2004 'Not A Pure Science: chemistry in the 18th & 19th centuries' Science:306:981-2.

[12] P. Drahos & J Braithwaite Sep. 2004 'Who Owns the Knowledge Economy: political organizing beyond TRIPS' Briefing Paper #32 by the Corner House, http://www.thecornerhouse.org.uk .

[13] As he put it: "Alle Ding sind Gift und nichts ohn Gift; alein die Dosis macht das ein Ding kein Gift ist." ("All things are poison and not without poison; only the dose makes a thing not a poison.").

[14] Beverly Purnell 18 July 2003 '(Introduction) To Every Thing There is A Season' Science:301:325 (et seq.--a collection of 5 papers on the role of timing during development, **plus** the Editorial (D. Duboile, 'A Time For Chronomics?') and several more 'timing of development papers on Science's site dedicated to biologic signal transduction knowledge environment, http://stke.org).

fungicide and a contaminant) responded in a *non-linear* D/R manner (replicates tested)[15]

Even the hormesis theory--that low doses do cause beneficial effects, which the chemical industry likes to emphasize[16]--only shows that biology is active at low doses. No surprise then that a developing flood of studies of low-dose toxicity show risk and the need for precaution, even though such low dose testing is still rare.

In fact, hormesis proponents typically cite the known beneficial health effects of ionizing radiation. A few paragraphs above, we mentioned two D/R relations of ionizing radiation. First, linear with no dose threshold (i.e. some damage occurs even at the lowest dose. Hundreds of studies confirm this model, thus it's the accepted wisdom for radiation's mutagenicity (DNA damage--usually to somatic, not hereditary (sperm or egg) cells) and its carcinogenicity. Second, radiation's low dose biologic effects go beyond zero harm to a beneficial effect.[17] But, contrary to what most hormesis proponents admit, there's already abundant evidence of a third shape of ionizing radiation's low-dose D/R curve: low, everyday X-ray doses are harmful to cultured human cell DNA, while higher doses are not (the mechanism may be that DNA repair enzymes do not detect low level of DNA breaks).[18] In fact, radiation is known to damage to the nucleus of bystanding cells that have not been struck, even after just a single ion has passed through the target cell's cytoplasm (not even its nucleus).[19] Low energy radiation is well-known to initiate complicated "charge transfers"l over long distances of double-strand (resting) DNA and the biomolecules in close association with DNA.[20] This low-dose harm is no suprise: life has even figured out how to use the very same solar radiation that damages DNA (high-energy ultraviolet (u.v.) range) to repair that damage: photolyase enzymes, which use near-u.v. solar radiation to catalyze their repair of the DNA damage![21] One hallmark of life has been its irrepressible vigor...a character certainly demonstrated by that adaption, no?!! The complexity of life's adaption to ionizing radiation contiues to be revealed; e.g. every second after a dose of radiation, a cell produces30 or so of the protein complexes (using dozens of genes) that is uses to excise & replace damaged DNA.[22]

As the tools for studying biology continue to improve, increaasingly more direct proofs of the foolishness of the 'dose makes the poison' assumption will flood in.

So, what is the evidence proving that*'the dose makes the poison'* is an unrealistic, unsafe basis to estimate chemical risk from?

First, the high, near-MTD doses of classic toxicology's chronic toxicity test protocols (still dominant today) are *so* high that they overwhelm much natural biochemistry, and so their results are not representative of our actual (and ubiquitous) low exposures. For example, an overhelming dose of any type of compound can induce cell division, a vulnerable period for damage to DNA (mutation), which is a leading cause of cancer and many other diseases).[23] Another proof (that high doses lead to cancer per se) from this author is that about half of the *natural* chemicals tested are carcinogenic at 1/2

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[15] Janine Ezendam et al. 2004 'Toxicogenomicas of Subchronic Hexachlorobenzene Exposure in Brown Norway Rats' Env. Health Perspectives:112:7:782-91. DNA microarrays are a burgeoning toxicology field, enabled by DNA replication enzymes which allow a rapid screen of the effect of changes (such as the application of a toxin) on a gene; this is a stimulus to test hundreds of different genes at once: a micro-array of genes.

[16] But instead of acknowledging this critical point, most hormesis proponents ignore it, in order to argue that hormesis proves that toxicology's safe dose determinations need to be raised (made less safe) A Dr. Calabrese (U. Mass./Ahmherst) is a leading hormesis researcher, but unfortunately he is also the leading crusador for this imprudent policy--e.g. note the dominance of industry advisors listed at his web site, http://belle.com.

[17] Jocelyn Kaiser 17 Oct. 2003 'Sipping From A Poisoned Chalice' Science:302:376-9 (see sidebar 'A Healthful Dab of Radiation?').

[18] Rothkamm K & Lobrich M. 2003 Apr 29 'Evidence for a lack of DNA double-strand break repair in human cells exposed to very low x-ray doses' Proc Natl Acad Sci U S A.:100(9):5057-62. **A commentary** in same issue overviews the developing data on low dose radiation's effects: W Bonner 'Low Dose Radiation: Thresholds, bystander effects & adaptive responses' PNAS:100:9:4973-5.

[19] Chumlin Shao et al. 14 Sept. 2004 'Targeted Cytoplasmic Irradiation Induces Targetted Responses' PNAS:101:37:13495-500.

[20] S Ray et al. 4 Jan. 2005 'On the Capturing of Low-Energy Electrons By DNA' PNAS:102:1:15-9; **and** Fangwei Shao et al. 28 Dec. 2004 'Long-Range Oxidative Damage to Cytosines in Duplex DNA' PNAS:101:52:17914-9.

[21] Alexandra Mees et al. 3 Dec. 2004 'Crystal Structure of a Photolyase Bound to a CPD-Like DNA Lesion After in-Situ Repair' Science:306:1789 et seq.

[22] M. Mone' et al. 9 Nov. 2004 'In Vivo Dynamics of Chromatin-Associated Complex Formation in Mammalian Nucleotide Excision Repair. PNAS:101:45:15933-7.

[23] Bruce Ames & Louis S. Gold 31 Aug. 1990 'Too Many Rodent Carcinogens: mitogenesis increases mutagenesis' Science:249:970-971. Ames is a highly distinguished mutagenicity (DNA damage) expert, but a skeptic on synthetic chemical causes of cancer.

their MTD, a very high dose.

*It is **critical not to over-extend** this argument , which has evidence to support it,to say that these chronic toxicity vertebrate assays are therefore completely unreliable (as those who make profit from synthetic chemicals often do)*. They suggest simply that the much lower doses experienced in actuality by people do not overwhlem biological systems and so wont lead to cancer and other chronic disease. That is, they ask us to simply assume that lower doses are not harmful, instead of asking for evidence of what happens to the delicate tissue of biology at these lower but universally-experienced doses.

Both sides in the debate about the reliability of the animal experiments for chronic effects agree that testing near the MTD is a poor compromise that can fail its purpose: to predict chronic toxicity from actual exposure levels. Ideally, near-MTD test doses would *not* be the basis to extrapolate the cancer potency of chemicals down to the realistic doses, or to assume that there is a no-effect threshold dose for non-cancer effects; yet, universally, they are. In short, Q-RA's fundamental failure to predict toxicity stems not from the classic chronic toxicity vertebrate assay itself, but from the assay's failure to test real-world doses.

In fact, it may be precisely this assay's long history of validity that is driving industry to call for its abolishment...when it's used in support of regulations![24] The chronic toxicity assay played a key role in demonstrating the hazards of DDT, benzene, vinyl chloride, and the cigarette tar, among hundreds of other chemicals it has tested. Yet industry still relies heavily on the chronic toxicity assay *in private*, using whatever dose range they are curious about. They seldom release their findings, even when they discover that chemicals cause harm in animals--for example, suppressing the hazards of vinyl chloride[25] (also see our summary of lab frauds). *It's a telling hypocrisy*: how can industry publicly argue that the whole-animal test is invalid, when they depend on it for their own purposes?

Amongst this swirl of economic, political and scientific arguments, it is helpful to segregate the "do high-doses predict risk?" from the "do animals predicit human risk?" questions.

There is some empirical data demonstrating that even the unrealistic high doses used can predict toxicity; in contradiction to the arguments of Dr. Ames and of industry, above. A study by the National Center for Toxicological Research tested an enormous number of animals at low environmental doses of some suspected carcinogens. The results mirrored the predictions of the standard high-dose (fewer animals) tests of those chemicals.[26] David Rall, former director of the Ntl. Toxicology Program (NTP, one of two gold standards on the planet for carcinogen tests), rebuts some of Dr. Ames' arguments. Rall points out that 90% of the time the same health effect occurs at the assay's somewhat lower test doses as does at the highest dose tested, often in a lnear D/R manner. More directly, only 5%-10% of the hundreds of chemicals tested so far at these high doses cause cancer,[27] so it is impossible that all compounds are carcinogenic at these doses (Ames' and industry's argument). Finally, the first two of the following studies showing that vertebrate test animals validly predict human risk, also indicate that high doses can validly predict lower exposures.

Otherwise, industry's argument to abolish the chronic toxicity test (for use in their regulation) depends entirely on proving that animals don't predict human risk. But the empirical evidence for this proposition appears non-existant, while there is overwhelming evidence against.[28] On theoretical grounds alone, test animals are vertebrates, as humans are; and from their genome-on-out they are largely (though not totally) identical to humans. And empirically, we know:

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[24] Center for Children's Health and the Environment 2002 'Animal Testing Works: We Should Listen to The Canaries', http://www.children's environment.org/position.htm#annimal; accessed 26 Feb. 2004 (a paper to document the claims made in a large advertisement placed in the New York Times by this professional center, located in Mt Sinai Hospital of NYC). Please note that the following few paragraphs of our text use some language directly from this document, without further attribution (simply because its arguments are perfectly congruent with ours).

[25] Bill Moyers 26 March 2001 'Trade Secrets' PBS TV investigation. To comprehensively review these chemical industry documents about animal studies and their suppression, visit www.ewg.org.

[26] Fundamental and Applied Toxicology (1983) 3:3. Journal of Environmental Pathology and Toxicology, Special Issue (1980) 3:3. 'Re-Examination of the ED01 Study' Fundamental and Applied Toxicology (1981) 1:1.

[27] Rall, D.P. 2000 'Laboratory Animal Tests and Human Cancer' Drug Metabolism Reviews:32:119-128; **also** Fung, V.A. et al. 1995 'The Carcinogenesis Bioassay in Perspective: Application in Identifying Human Cancer Hazards' Environ. Health Perspectives:103:680-683.

[28] E.g.: Huff, J. 1993 'Chemicals and Cancer in Humans: First Evidence in Experimental Animals' Environmental Health Perspectives:100:201-210. **And** Rall, D.P. 1995 'Can Laboratory Animal Carcinogencity Studies Predict Cancer in Exposed Children?' Environmental Health Perspectives:103 suppl. 6: 173-175.

- About one-third of *known* human carcinogens were first discovered through animal studies.[29]
- Every known human chemical carcinogen is also an animal carcinogen.[30]
- Typically carcinogens cause cancer of the same organs in both animals and humans.[31]
- Almost every known chemical cause of human birth defects--98%--also cause birth defects in animals.[32]
- Every known chemical that disrupts human hormonal function does the same in animals. Conversely, when tests show that chemicals harm animals, those chemicals almost always cause similar harm in humans.[33]

Of course, despite many similarities, animals do differ from humans. About 25% of the compounds that cause cancer in mice do not in rats[34] (the liver peroxisome proliferation mechanism of DNA damage is one reason). This is why chemicals are considered hazardous only after they have been shown to cause harm in two species, for example, mice and dogs, or rats and monkeys. As the number of species that suffer harm from exposure increases, the likelihood of that compound being a hazard to humans also increases.[35]

Rall also shows that chronic toxicity animal tests have given very low false positive results (while false negatives are very hard to evaluate); and that every one of the few chemicals established as a human carcinogen (31 chemicals at that point) has also proven to be as a carcinogen in these animal tests. Finally, he briefly reviews accumulating evidence that similar cancer responses to test chemicals occur between vertebrates (though some different responses exist too).[36]

Another part of this debate between Ames' "don't worry" message and scientists more concerned about synthetic chemicals examined Ames' argument that natural chemicals are more potent carcinogens than industrial chemicals. This brings up the question of evolution and more recent response to the presence of natural and (very recently) synthetic chemcials that are carcinogens in the rodent assays. A correspondance exchange to Ames' argument establishes at a minumum that human diets have varied for at least 4 million years, and cooking has gone on for 700,000 years. Thus there's been time to adapt to various natural carcinogens, and examples of such adaptation are given. The other argument, that adaption can only be passed on if the expposure to natural carcinogens occurs before the end of reproductive life, is obviously only half an argument.[37]

Finally, beyond the two “gold-standard” cancer assays (the USA government’s National Toxicology Program and the World Health Organization’s Int’l Agency for Research on Cancer), there exists a third, the European Ramazinni Foundation for Oncology and Env. Sciences, Bologana Italy; with a reputation of independence and objectivity. They observe their animals until ‘natural’ death, rather than killing them shortly after the end of dosing; allowing a chance for chemical-induced cancers and other diseases to develop! Since 1966 they have found unequivocal evidence of carcinogenicity in 23.5% of the 200 chemicals tested as of 2002,[38] and obviously therefore many more of those 200 chemicals showed some evidence of cancer. They also imply that such independent and objective animal assays will often find positive results where previous less-rigorous assays did not (such as those by those who sell the chemical); citing their positive results for vinyl chloride, benzene, formaldehyde, MTBE (the gasoline oxygen additive). The

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[29] Huff, J. 1994 'Chemicals Causally Associated with Cancers in Humans and in Laboratory Animals: A Perfect Concordance' in M.P. Waalkes and J.M. Ward (eds.) Carcinogenesis (Raven Press, NY) pp. 25-37.

[30] Rall 1995; **also** Huff 1993.

[31] Huff 1994.

[32] Klaasen, C D 1996; **also** Kimmel, C. et al. 1992 Animal Models for Assessing Developmental Toxicity: Similarities and Differences Between Children and Adults. (ILSI Press, Washington D.C.) pp. 43-65.

[33] LeBlanc, G.A. 1995 'Are Environmental Sentinels Signaling?' Environ. Health Perspectives:103:888-890; **also** Colburn, T. 1994 'The Wildlife-Human Connection: Modernizing Risk Decisions' Environ. Health Perspectives:102(suppl 12):55-59; **also** Couch, J.A. and J.C. Harshbarger. 1985 'Effects of Carcinogenic Agents on Aquatic Animals: An Environmental and Experimental Overview' Environmental Carcinogenesis Reviews:3:63-105; **also** Huff 1994 pp. 25-37; **also** Guillette, L.J. 1994 'Endocrine-Disrupting Environmental Contamination and Reproduction: Lessons from the Study of Wildlife' in D.R. Popkin and L.J. Peddle (eds.) Women's Health Today: Perspectives on Current Research and Clinical Practice (Parthenon, Pearl River, NY) pp. 201-207.

[34] Huff, J. 1991 'Chemicals Associated with Site-Specific Neoplasia in 1394 Long-Term Carcinogenesis Experiments in Laboratory Rodents' Environ. Health Perspectives:93:247-270.

[35] Alden, C.L. et al. 1996 'A Critical Appraisal of the Value of Mouse Cancer Bioassay in Safety Assessment' Toxicology Pathology:24:722-725; **also** Maronpot, R.R. and G.A. Boorman 1996 'The Contribution of the Mouse in Hazard Identification Studies' Toxicology Pathology:24:726-731.

[36] Rall 1991.

[37] Devra Lee Davis ?8 Dec. 1987 'Paleolithic Diet, Evolution & Carcinogens' (correspoondance) Science:27?:1633-4; **and** response by B. Ames & L. Gold, p. 1634.

[38] M. Soffritti et al. 2002 ‘Ramazzini Foundation Cancer Program: history & major projects…’ Ann NY Acad Sci:982:26-45.

ubiquitous soft-drink sweetener aspartame recently joined that list.[39]

In sum, if the whole-animal long-term toxicity assay itself is evidently not the problem; what can be demonstrated about the predictive validity of 'high to low dose result extrapolation'? A paper cited by Ames & Gold themselves (in their correspondance exchange with Rall) actually argues persuasivelly (given its early date) that low environmentally relevant dose carcinogenesis is more likely to be caused by agents that cause cell proliferation via a cellular receptor (such as many hormones), and also sometimes from those that cause mutations to DNA; but not from agents that cause cell proliferation un-mediated by a cell receptor (i.e., tumors from high-dose induced mitosis).[40] Thus they themselves cite evidence for serious disease from low doses!

Even though some evidence (above) indicates that the standard high-dose chronic test may reliably predict risks from environmentally relevant doses, it actually often substantially underestimates human risk, according to the National Research Council.[41] Why this might be so is our next, and more important, discussion.

Second (as to why 'the dose makes the poison' is an unsafe safety test paradigm), direct evidence of low dose harm. *The above fascinating scientific debate about the validity of the results of high doses is obviously not as important as the question that it dances around: are our actual exposures--low dose yet ubiquitous; i.e. 'environmentally relevant'--causing harm?*

Biology is nothing more than chemical signals, almost always very complex.[42] Un-surprisingly therefore, observation has already shown that hundreds of biochemical signals occur at extremely low concentration of the endogenous or exogenous chemicals that cause it; thus far, in a nascent field.[43] Biology happens at such low levels of signal transduction that a single low, ubiquitously-encountered, exposure to a toxin may alter it; many examples follow.

These biochemical signals are proving to frequently occur at very low-doses and in non-linear dose-response relations. Because of this subtlety and complexity of biology, chemicals (presumably especially ones that biology did not evolve in close proximity with, such as the unusualy strong halogen-to-carbon covalent bond or the arrangement of protons, neutrons & electrons of the toxic heavy metals)--at low enough doses that they are ubiquitous--have a high potential to affect biology. After all, biology is chemicals.

But the chemistry that is life is organized into hyper-sensitive systems, indeed! For example, biological signals come with noise (non-signal). Noise is either intrinsic, from the biochemical reaction being studied; or extrinsic, from adjoining biologic activity. Just as in electronics, when there is high amplification (i.e. a low-dose signal stimulates a lot of activity), noise increases in proportion to the amplification rate, and it tends to come from extrinsic sources. But if a highly amplified signal is emitted at a rapid frequency, noise tends to be filtered out.[44] Yet these authors note that low noise is not always a good thing, as it limits learning and physical evolution. Extrinsic noise is known to be controlled, but it has been modelled thatfactors such as intrinsic delays in biologic signals can cause that feedback system to lose equlibrium.[45] Such are the everyday complexities of biology.

The authors caveat that these rules may not apply to non-linear high-amplification signals. But non-linear responses are apparently common at ultra-low doses of signaling,[46] a review of the scientific literature found over 100 studies featuring

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[39] M. Soffritti et al. 2005 'Aspartame Induces Lymphomas & Leukemias in Rats' Europn J Oncol:10:2:?.

[40] S. Cohen & L. Ellwein Aug. 1990 'Cell Proliferation in Carcinogenesis' Science: 249:1007-1111.

[41] Bucher, J.R. 2000 'Doses in Rodent Cancer Studies: Sorting Fact from Fiction' Drug Metabolism Reviews:32:153-163; **also** Nat. Research Council 1993 'Pesticides in the Diets of Infants and Children' National Academy Press, Washington, D.C.; **also** Rice, D. et al. 1996 'Lessons for Neurotoxicology from Selected Model Compounds: SGOMSEC Joint Report' Environmental Health Perspectives:104:205-215.

[42] D. Koshland 8 May 1998 'The Era of Pathway Quantification' Science 280:852-853. This journal now has a strong focus on low-dose biology signals, continually publishing news and research, and formed the Signal Transduction Knowledge Env.--a new journal http://skte.org--to keep non-specialists current due to the complexity of this field. See Science 31 May 2002, the editorial and p. 1632 et seq.: "Please explore the beauty and complexity of these pathways...".

[43] Koshland 1998 (e.g.).

[44] Tatsuo Shibata & Koichi Fujimoto 11 Jan. 2005 'Noisy Signal Amplification in Ultrasensitive Signal Transduction' PNAS:102:2:331-6.

[45] D Bratson et al. 11 Oct. '05 'Delay-Induced Stochastic Oscilations in Gene Regulation' PNAS:102:41:14593-8.

[46] Koshland 1998.

non-linear signals,[47] again despite this being a very new field of study.

The young field of endocrinology (hormone) disruption ,too, already knows of hundreds of ultra-low dose toxicity results in all manner of vertebrate species, including mammals.[48] In endocrinology, non-linear D/R curve results are so common that they are seldom even submitted for publication![49] Welshons et al. test the 'spare receptor hypothesis'--that species evolved the ability to respond to low levels of hormones (ligands)in their bodies--and shows that to be the case for several estrogenic chemicals. *In other words, estrogenic effects (throughout male and female bodies) were initiated more easily at lower doses of the estrogenic chemical than at higher doses*. In fact, among their extensive experiments, the high ppm doses typical of chronic toxicity tests for estradiol (E2, the active form of estrogen) were less proliferative on cells than even the negative control(!), but highly proliferative at low, real-life doses (from high parts per quadrillion to low ppb). And while E2 is more potent than many industrial chemicals that are estrogens, there is copious data (some summarized in this paper) showing that actual body burdens of industrial hormones cause adverse hormonal effects. *Biologic events downstream of receptor-binding are even more sensitive*. Welshons et al. emphasize that the threshold-dose assumption (that no effect is possible below a very high, assumed no-effect dose) employed in non-cancer chronic toxicity tests causes a large-to-infinite error, since--for estrogens and other hormones at least--the body is designed to repsond at endogenous hormone concentrations. Similarly in brain devvelopment and function, hormones are potent at doses far, far below those used by chronic toxicity tests, and turn off (receptor production is shut down) as doses rise from these very low levels.[50]

A crucial underestimate of toxicity is that very often (e.g. almost every hormone toxicity test) is to assume that toxic effects require the toxin to reach the nucleus and act on the genome. Many toxins including hormone mimics are indeed so transported and over hours or days to (perhaps with continuing exposure) the disturbeded genome expresses an amazingly diverse spectrum of toxic effects (including much chronic disease). However, attention is just now turning to far more rapid low-dose toxicicity caused when toxins first interact with cells: *at the cell membrane*. Docking to receptors in the membrane initiates a complex chain of signals designed to amplify very low concentrations and rapidly communicate commands to the genome, so as to execute all-important functions such as replication, differentiation, stress response and more--all functions intimately related to chronic disease. These powerful signal chains are now being made visible.[51] Thus, even a single molecule of a toxin may rapidly elicit a strong inappropriate biologic signal, and continuing such an improper signal may lead to terrible effects.

An extensive set of experiments with hormone disruptors not only found that every one of these synthetic toxins rapidly (less than 3 minutes) disrupted a variety of important membrane-initiated signalling cascades, in some cases these important signalling cascades were severely disrupted at xenoestrogen concentrations in the low picoMolar range (roughly, low parts per quintilion).[52] Similarly, the potent synthetic chemical bisPhenol-A (used in some pesticde formulations, inter alia) uses membrane receptors to very rapidly disrupt insulin function.[53] Many other studies show vast and nearly instant amplification of signals inputed at the membrane. *Once again it is proven that toxins cause severe disruption of life at commonly experienced yet never tested doses.* In fact a low dose signal at the membrane by one hormone (T3-thyroid) causes in the nucleus even lower concentration of a *different* hormone—estradiol—to begin transcription of genes[54] (a process apparantly more rapid than the traditional route to gene transcription).

The activity of many proteins are closely regulated by other molecules, often by a small molecule that alters the shape of a protein/receptor compex ("cooperative binding"). It is known that often a small change in the concentration of these regulator molecules elicits dramatic changes in the function of the protein. RNA too is so regulated, thus gene expression too is obviously subject to such delicate control.[55] Finally, the sensitivity of organisms to hormone-receptor signaling

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[47] Environmental Health Perspectives Oct. 2001, 109:10:1063-1:070. (EHP is the peer reviewed journal of the National Institute of Env. Health Sciences (NIEHS), part of the Ntl. Institutes of Health).

[48] Ntl. Toxicology Program (NTP) 2001 'Report of the Endocrine Disrupters Low-Dose Peer Review' Wash. DC.

[49] W. Welshons et al. 2003 'Large Effects Fron Small Exposures. I. mechanisms for endocrine disrupting chemicals with estogenic activity' Env. Health Perspectives:111:6:994-1006 (doi: 10.1289/ehp5494).

[50] Theodora Colborn June 2004 'Neurodevelopment and Endocrine Disruption' Env. Health Perspectives:112:9:944-9.

[51] Bharath Ananthanarayanan et al. Oct. 18 2005 'Signal Propagation From Membrane Messengers to Nuclear Effectors Revealed by Reporters of Phosphoinositide Dynamics and Akt Activity' PNAS:102:15081-15086.

[52] Natalyia Bulayeva and Cheryl Watson Nov. 2004 'Xenoestrogen-Induced ERK-1 and ERK-2 Activation Via Multiple Membrane-Initiated Signaling Pathways' Env. Health Perspectives:112:15:1481-7 (doi: 10.1289/ehp7175).

[53] Paloma Alonso-Magdalena et al. Aug. 2005 'Low Doses of BisphenolA & DES Impair Ca2+ Signals in Pancreatic a-Cells through Non-Classical Membrane Estrogen Receptor' Env Health Perspec.:113:8:969-77.

[54] Xing Zhao et al. 29 Mar. '05 'Thyroid hormone can increase estrogen-mediated transcription…' PNAS:102:13:4890-5.

[55] M. Fanulok 8 Oct. 2004 'RNAs Turn On In Tandem' Science:306:233-4 (a Perspective on the paper: M. Mandal et al. 'A Glycine-Dependent Riboswitch That Uses Cooperative Binding to Control Gene Expression', in the same issue).

causes additional false negative test results, due to the difficulty in controlling the dose of hormonal chemicals--hormonal agents in feed, bedding, even in plastic cages can add to the assumed test dose and reach the dose that shuts-off the response. Even the breeding of test animals for fecundity (e.g. the popular CD-1 mice) has been shown to underestimate the effects of hormones.[56]

Shocking new data has just been published that highlight how the subtleties of biology dictates complex dose/response relations (not the asinine linear and threshold D/R curves of Q-RA). In non-cancer Q-RA, EPA has begun to look at responses to both wider and more finely-resolved dose ranges, substituting a methodology called benchmark dosing (BMD) in place of the--alleged--NOAEL basis for determining non-cancer RfDs and RfCs. BMD testing requires both broader and more resolved dose ranges, at least enough so to be able to determine at what point a response significantly differs from normal (typically 1% to 10%), called 'Point of Departure (PoD). Detecting a 1% to 10% PoD also typically requires more test animals than used in a NOAEL test.

For the couple of dozen industrial chemicals for which EPA so far has validated test results using both methodologies, EPA and UC/Berkley authors found that when the dose is their previously-determined RfD or RfC, most of the chemicals had lifetime non-cancer risks of 1-in-200 to 1-in 10,000 (of the specific end-point observed)![57] I.e. when realistic dose ranges are actually tested instead of extrapolated to, the "reference" (i.e. safe) dose (RfD or RfC) that all industry and all governments have assured us is absolutely safe, is actually extremely risky!! [This, assuming that only a greater than 10% change in the endpoint measured is significant. But more important is that even a complete dosing regime will not suffice to estimate risk until we are able to measure the thousands of distinct biologic operations of complex organisms (Problem #4 below)].

A further layer of the subtlety of signaling (of biology), and an example of the timing of an environmental insult being more important than its dose (Problem #3, below)is that these signaling systems may display learning. The apparently exact same environment (including the input to the signal, e.g. a dose), may elicit a different signal (response) in a cell, depending on its history. Learning--a complex behavior for a physical system--is used by cellular systems to create positive and negative feedback loops and to create logic devices, such as ‘either/or’ gates.[58] Finally, it appears that signal co-activating proteins do not vary in concentration in most cell types, therefore other variables (e.g. timing, synergy), not just the dose, have to play a key role in the transduction of many biological signals.[59]

Also, a thorough analysis of all neurotoxicity test results of four known neurotoxic chemicals/families (Pd, Hg, PCBs and solvents) concluded that the regulatory neurotoxicity tests for all these compounds failed to find known neurotoxicities, which often occured three orders of magnitude (a thousand times) below the doses used in the regulatory tests (a key reason was failure to test during development, a more vulnerable period--see Problem #3 below).[60]

Further, organisms have various systems to detoxicfy dangerous compounds. Yet it was recently shown that one of these systems is inhibited by the very toxins it's designed to protect from! The MultiDrug/Xenobiotic Resistance efflux transporter (MD/XRs, which bind to and eject dangerous molecules before they fully enter cells--i.e. from the cell membrane) system is designed to be very non-specific. An experiment with synthetic musk compounds (used as fragrances and fixatives in personal care products) showed that the musks inhibited the ejection of other foreign molecules from cells for up to two days, after a just two hour exposure to musk concentrations of just 10 to 100 times higher than average musk levels found in humans.[61] Because MD/XRs are especially attracted to lipophillic compounds, i.e. toxins that are persistant and bioaccumulate, the critical MD/XR detoxification system may be significantly disabled by existing body burdens of such toxins.

Another review finds high-quality evidence of toxicity from commonly-encountered exposure levels of common pollutants (lead, environmental tobacco smoke, radon and drinking water disinfection by-products); risks which are not assessed in setting limits (e.g. bladder cancer from drinking water with just 1/80th the level of trihalomethanes such as chlorform that

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[56] J. Spearow et al. 20 Aug 1999 'Genetic Variation in Susceptibility to Endocrine Disruption By E.in Mice' Science:285

[57] Rosemary. Castorina, Tracey Woodruff Aug. 2003 'Assessment of Potential Risk Levels Associated with U.S. Environmental Protection Agency Reference Values' Environmental Health Perspectives:111:10:1318-1325.

[58] U. S. Bhalia and E. M. Machleder 2002 Science:297:1018. See also the news story about this paper in the same issue, pp. 948-9 (N. Ingolia and A. Murray, History Matters’).

[59] McDonald, Norris 31 May 2002 ‘Connections & Regulation of the Human Estrogen Receptor’ Science:296:1642-44.

[60] Rice et al. Apr. 1996 'Lessons for Neurotoxicology From Selected Model Compounds: SGOMSEC Joint Report' Env Health Persp.:104:suppl.2:205-12.

[61] Till Luckenbach & D. Epel Jan. '05 'Nitromusk & Polycyclic Musk Compounds As Long-Term INhibitors of Celular Xenobiotic Defense Systems Mediated by MultiDrug Transporters' Env. Health Perspectives:113:1:17-24.

the EPA allow in drinking water).[62]

Given all these (and many more) examples of low dose toxicity, it should be no suprise that, among herbicides alone, 60% are already documented to be hormone disruptors (without any hormone screening program--likely almost all chemicals are hormone disruptors).[63] Of many other examples that we are aware of, several ***pesticide-specific*** ones illustrate the potent response of biology confronted with low-dose (real-world) levels of toxic chemicals exogenous to it. :

> A concentration of just four to six or so *molecules* per cell of several potent estrogenic agents (such as some pesticides) in male rodent prostate cells triggers those cells to multiply inappropriately, but increasing the concentration a little shuts off that effect.[64] One likely mechanism for such a result is that too many hormones in a cell are known to turn off the production of the receptor molecule that activates them.[65] Uncontrolled cellular division is called hyperplasia, a necessary step for cancerous tumors. In fact, prostate cancer is epidemic in industrialized countries.[66]
>
> The carcinogenic, persistent fungicide and herbicide contaminant hexachlorobenzene (HCB, allowed at up to 200 ppm in picloram--the most popular herbicide on public lands); already associated with several human reproductive disorders; was shown to significantly speed sexual maturity of the prostate in male mice at a low environmentally relevant dose; yet it significantly retarded this development at doses just two to over 20 times higher. In vitro, it was confirmed that the low dose HCB stimulated action via the androgen hormone receptor while high dose repressed it.[67]
>
> Trimec (a mixture of three related herbicides--2,4-D, mecoprop and dicamba, each planned for use in this FEIS--plus the other formulated ingredients) was added to the drinking water of gestating mother mice. The authors used EPA's reproductive effect test protocol for pesticide registration but added a lower dose--altogether the four doses spanned a 10,000-fold range. At several times of the year (because season affects sex hormone production in mothers, a variable), up to a 20% increase in failed pregnancies resulted; but as the dose increased, the effect lessened--at every dose level; and smoothly. Almost as alarming, the lowest dose (i.e. that which showed the greatest toxicity) of the 2,4-D within the mixture was seven times lower than the maximum EPA allows in drinking water. That dose was selected because it is equivalent to EPA's RfD--the supposedly "perfectly safe" dose--for 2,4-D.[68]
>
> The ubiquitous chemical bisPhenol-A (inter alia, an ingredient in some pesticide formulations), in the range of typical human exposure levels, causes exposed mice fetuses to gain excessive weight during life, a major risk factor for many deadly diseases. Moreover the effect was greatest at the lower doses tested.[69] Obesity rates are well-known to have exploded in recent decades in industrial countries, although it is obviously a multi-factorial syndrome. bPA is an estrogen so unsuprisingly it has many other toxic low-dose effects not exhibited at typical test doses (and two industry papers finding no low-dose toxicity are shown to be invalid[70]). It has also been

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[62] Wigle DT & Lanphear BP (2005) Human Health Risks from Low-Level Environmental Exposures: No Apparent Safety Thresholds. PLoS Med 2(12): e350.

[63] P. Short & Theodora Colborn 1999 'Pesticide Use in the U.S. and Policy Implications: a focus on herbicides' Toxicol. & Ind. Health:15:240:75.

[64] F. Vom Saal 2000 at the Ntl. Coalition Against Misuse of Pesticides (NCAMP, now Beyond Pesticides) annual conference (videotaped presentation). **Also**: F. Vom Saal et al. 2002? 'A Physiologically-Based Approach to the Study of bisphenol-A and Other Estrogenic Chemicals On the Size of Reproductive Organs, Daily Sperm Production & Behavior' Toxicol. & Indus. Health:1&2:239-260; **and** F. Vom Saal et al. 1995 Toxicol. Ltrs. 77:343-350; **and** F. Vom Saal et al. 1997 'Prostate Enlargement in Mice Due to Fetal Exposure to Low Doses of Estardiol or Diethylstilbesterol, and Opposite Effect at High Doses' Proceedings Nat. Acad. Sciences 94:2056-2061.

[65] Louis Guillette Jr. 2000 NCAMP annual Conference (videotape). Dr. Guillette is an experienced endocrinologist.

[66] Ntl. Cancer Institute (NCI) 1994 'SEER Cancer Statistics Review, 1971-1991' NIH Pub. No. 94-2789, Bethesda MD.

[67] Jody Ralph et al April 2003 'Disruption of Androgen Regulation in the Prostate by the Env. Contaminant Hexachlorobenzene' Env. Health Perspectives 111:4:461-466.

[68] M. Cavieres et al. 2002 'Developmental Toxicity of a Commercial Herbicide Mixture in Mice: I. effects on embryo implantation and litter size' Env. Health Perspectives 110:11:1081-1085. Recently Dow Inc., BBI Sciences Inc. and the Industry Task Force-II on 2,4-D Research; and Syngenta (another herbicide manufacturer), in published correspondance (EHP:111:9:A450-451, July 2003) **argue** that the study should be **retracted** due to alleged problems with the experiments, which they lay out. The **authors rebut** in detail this industry letter in EHP:111:14:A748-751, Nov. '03.

[69] Bev. Rubin et al. July 2001 'Perinatal Exposure to Low Doses of bisPhenol-A Affects Body Weight, Patterns of Estrous Cyclicity and Plasma LH Levels' Environmental Health Perspectives:109:7:675-680.

[70] Welshons et al. 2003.

found a dose of 1 mg/kg of bP-A activates the estrogen receptor in live mouse foetuses, yet it does not in the in vitro tests that are ubiquitously used to test for estrogenicity.[71] However it has shown various toxicities at doses that range a million-fold(!); and while the current regulatory "safe" dose is 1/50th of bPA's maximally tolerated dose, it is toxic at 1/25,000th the MTD (e.g. it disrupts cell function at 0.23 pptrillion).[72]

It appears that the massive-use, highly water soluble herbicide atrazine, an established hormone disrupter, may cause stronger effects at actual exposure low doses than at the higher doses of toxicity testing. Developmental endocrinologist Tyrone Hayes (UC/Berkley) has shown, both in the lab and in actual field conditions, that developing male tadpoles in water with 0.1 ppb to less than 1.0 ppb concentration of atrazine became demasculized (including depressed testosterone) and hermaphroditic. The 0.1 ppb dose--a ubiquitous environemntal concentration, i.e. even in areas where it is not used--is 30,000,000 times(!) lower than the result using the traditional protocol for amphibian reproductive toxicity. A 25 ppb concentration in the lab, or uncontaminated field frogs, did not elicit/show those effects.[73] Hayes believes atrazine may be inducing the production of the enzyme aramotase, used to produce the correct male and female sex hormone ratios, a key to sexual differentiation (all vertebrates begin female). His team repeated his two sets of experiments four times resulting in replicating results 51 times. Meanwhile scientists funded by Syngenta, the prime manufacturer of atrazine say they are unable to replicate this effect.[74] Despite these alarming results at up to 30 times below the allowable level of atrazine in drinking water (3 ppb), EPA just declined to change that required-to-be safe exposure level, and in re-registration imposed no significant restricitons on atrazine use.

In extensive experiments for both low-dose and synergy effects, rats dosed with the pesticide chlordecone and the solvent/cleaner carbon tetrachloride (significantly below acutely toxic levels) caused a 67-fold increase in liver toxicity, but not complete liver failure; while substituting phenoparbitol for carbon tetrachloride in the mix caused complete liver failure, even though it caused half as much liver damage as the first mixture! Extensive experminents indicated that this and similar results are likely due to tissue repair programs being activated by tissue damage, but unpredictably.[75] They call this a 'two-threshold doses' model of toxicicity and point out the serious challange it poses to traditional Q-RA. We say such results lay waste to the linear dose/response assumption that toxicology relies so completely on!

In elegant experiments using mainly pesticides, it was shown that for activated signalling systems (such as a hormone docking with its receptor) there may be no dose threshold below which no effect occurs.[76]

Various combinations of the herbicide atrazine, the insecticides aldicarb and carbamate, and nitrate (fertilizer) had synergistic effects...at the concentrations they're typically found in groundwater--below drinking water limits.[77]

Atrazine is yet again shown to be toxic (the long-term survival of tadpoles) at a low, everyday-exposure dose (3 ppb--the current EPA drinking water limit); but this time the low dose was also more toxic than higher doses (30, 100 ppb--typical of pulses received by waters).[78]

Metalaxyl is a fungicide with arylamide impurities (a family of mutagenic and carcionogenic nitrogenated organics, common in meat char, cigarrette smoke, permanent dyes and alumininum production)--mainly with 2,6-

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[71] Josephine Lemmen et al. Nov. 2004 'In Vivo Imaging of Activated Estrogen Receptors In Utero By Estrogens and Bisphenol A' Env. Health Perspectives:112:15:1544-9.

[72] F vomSaal & C Hughes Aug. 2005 'An Extensive New Literature Concernincg Low-Dose Effects of Bispehnol A Shows the Need for a New Risk Assessment.' Env Health Persp:113:8:926-33 (Commentary).

[73] T. Hayes et al. 2002 'Hermaphroditic, Demasculized Frogs After Exposure to the Herbicide Atrazine at Low Ecologically Relevant Doses' Proceedings Ntl. Academy of Sciences:99:5476-5480 (lab experiment). **And**.Hayes et al. April 2003 'Atrazine-Induced Hermaphroditism at 0.1 ppb in American Leapord Frogs (Rana pipiens): Laboratory & Field Evidence' Env. Health Perspectives:111:4:568-575.

[74] R. Renner 2002 'Conflict Brewing over Herbicide's Link to Frog Deformities' Science 1 Nov. '02, p. 941-2.

[75] M. Soni & M. Mehendale 1998 'Role of Tissue Repair in Toxicologic Interactions Among Hepatic Organics' Env. Health Perspectives:106(Suppl.6):1307-1317.

[76] D. Sheehan et al 1999 'No Threshold Dose for Estradiol-Induced Sex Reversal of Turtle Embryos' Env. Health Perspectives:107:155-159.

[77] W. Porter et al. 1999 'Endocrine, Immune & Behavioral Effects of...At Groundwater Concentrations' Toxicol. & Industrial Health:15:1&2:133-150.

[78] Sara Storrs and J Kiessecker July 2004 'Survivorship Patterns of Larval Amphibians Exposed to Low Concentrations of Atrazine' Env. Health Perspectives:112:10:1054-7.

dimethylaniline (2,6-DMA). "Extremely low environmental concentrations" of 2,6-DMA metabolites were recently associated with non-smoking bladder cancers. But much of human exposure may come from 2,6-DMA being the metabolite of the common drug lidocane and the veterinary drug xylazine.[79]

A fascinating example of unexpected, wildly non-linear D/R is that the potent develpmental hormone retinoic acid (RA) (which various pesticides and some other cynthetic chemicals mimic) at a lower dose caused more left eye underdevelopment in frogs than a higher dose; but the higher dose caused more underdevelopment of the right eye![80] Many synthetic chemicals mimic or disrupt RA and its necessary binding to its receptor.

The third and final proof that 'the dose does *not* make the poison' is: "Complexity is easy to generate, much harder to control"[81] --**e.g., Epigenetics**

If you've ever observed any of the extraordinary adaptations by species (e.g. the horns of various beetle species), perhaps you've wondered, as we have: how on Earth did small, advantageous random mutations manage to--in just the few hundred million years since multicellular life began--lead to such diversity, and to such exquisite adaptions, that life displays today?!

And, how is it that life evolved from relatively simple, undifferentiated replicating cells; to the metamorphosis--phenomenally complex and exquisitely differentiated--that marks the development of most species multicellular species; a journey that begins as such an undifferentiated single cell (e.g. from a simple and tiny seed to a sequoia tree or human)? --

Obviously, complexity is easy to generate (we see it all about us, both natural and human-caused); and, just as obviously, it is harder to organize and to control it than it is to create it. As summarized in Mattick 2004, life did not much diversify until after eukaryotic cells evolved (cells with a true nuclei for genetic material and a complex outer membrane; unlike the earlier prokaryotic cells, e.g. bacteria). More complexity requires ever more organizing of systems (beyond the programming of individual components). It is possible that eukaryotic cells--the first to centralize the functions of heredity into a cell nucleus--were necessary before more varied species could be created.

The individual molecules of biology are identical across much of life--e.g. 99% of the proteins of mice have a recognizable equivalent in humans. Rather, it's the *organization* of these mass-produced components that causes complexity and diversity--i.e. the different activity of the human's vs. the mouse's identical proteins.[82] Organization (including networking) that self-executes is the key, says Mattick.

Now there is accumulating evidence (follows) that in allowing diversity, eukaryotic cell's centralized genetic material also controlled and organized that genetic complexity. Another line of evidence also points to the need of eukaryotic cells to evolve before such complexity can be regulated: the largest known genome of a prokaryotic cell is about the theoretical amount of DNA needed to create the maximun amount of protein-coding genes without a lot of regulatory components.[83]

In fact, today's theory of evolution--for now prosaically called *epigenetics* ('epi' = "over", "around", "beside" a gene)**--posits a transformed theory of genetic control of cells, one that is rapidly unraveling the too-simple 50 year-old central paradigm of biology**.

That organizing paradigm of biology, for the half-century since just after the seminal 1954 discovery of the chemical and physical structure of the DNA double-helix (which composes the chromosones, then known to control reproduction and heredity, and suspected to control many other functions, but little was known about the mechanism) was known as the: 'One gene, one protein' theory. It is a transcription-based theory of gene expression, positing how genes command biologic activity (a protein--the end product in the original theory of gene transcription--is made up of certain amino acids; see below).

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[79] Tracy Hampton 3 Nov. 2004 'Chemicals Linked to Bladder Cancer in Smokers May Play Wider Role' JAMA:292:17:2072; **referencing:** Jinping Gan et al. 6 Oct. 2004 'Alkylaniline-Hemoglobin Adducts and Risk of Non-Smoking-Related Bladder Cancer' JNCI:96:19:1425-31.

[80] D. Alsop et al. 'Dietary Retinoic Acid Inbduces Hindlimb & Eye Deformities in X. laevis' Env Sci & Technol:38:23:6290-9 (15 Nov '04).

[81] J Mattick Oct. 2004 'The Hidden Genetic Program of Complex Organisms' Scientific American:60-7.

[82] Mattick 2004.

[83] Mattick 2004.

That original 'one gene, one protein' theory of gene transcription (expression) was as follows. First, genes are a sequence (a few dozen to a few hundred) of the four bases of DNA (adenine, guanine, cytosine and thymine, the alphabet of the instructions for all life on Earth; linked in a strand. For storage and based on their electrical charges, the strands pair into two complementary (oppositely charged) sets (C-G, A-T), to create the famous double-helix of two long DNA strands (every human cell has almost two meters of double-helix, tightly wound into 23 chromosones that occupy much of the nucleus). Start and stop areas define the extent of each gene on the DNA strand, with the large remainder originally thought to be "junk" or "non-sense"--useless--DNA (in fact, almost 99% of each cell's 3.1 billion base pairs in a human is not our 30,000 or so classic genes--about the same number as much less complex species, e.g. fruit flies). A good portion any organism's DNA is the genes and other command elements of other organisms that every species picks over its evolution; therefore humans are far more deeply connected to and every other species and to the biosphere than most of us realize.

Next in the classic theory, a strand of the double-helix is unwound, locally, to allow the transcription of a gene. A strip of complimentary (to the DNA--i.e. opposite charges) transcription RNA is built from enzymes (catalytic proteins) and other proteins that clamp on to the DNA, read it, replicate the essential part of the base, and move to the next base. As the DNA double-helix is re-joined and rewound onto its chromosone, the new RNA sequence moves outside of the nucleus and translated into a protein.

Most codes (letters) are more manageable as words. If the words given by the genetic code were two bases long, there would only be 12 possible unique combinations, a somewhat limited foundation from which to build the hundreds of thousands of proteins (sentences). Words of 3 letters, however, allow up to 64 unique sequences of the bases to build proteins from. And in fact, life on Earth did adopt three base-long words. So, from the RNA's transcribed sequence, three new bases (manufactured separately) at a time are strung together in chains. Finallly, these chains of words (sentences) fold naturally into their final pre-designed (due to the location of the molecule's electrical charges) 3-D shapes. We call these sentences proteins, and their words (building blocks) are called amino acids. The shapes of proteins, and the final electrical charges they carry, do almost all the work of life.

All life on Earth uses just 20 words (amino acids, or 'codons') from the genetic alphabet of the four bases, despite there being 64 unique three-base long combinations of the four letters available. Despite the near-universality of this 20-word language across all species, it now appears that these 20 words are not immutable. An occassional species uses a new word (via mutations). Nor does it appear that these 20 words were randomly selected: these occassional variations of the language of life may be used by those species to limit more powerful dangerous mutations.[84]

Note that even had this original simple theory of the origin of biochemical commands *perfectly* explained all signals to cells, chemical Q-RA would *still* fail to identify even the known risks of gene disruption by synthetic chemicals and other un-natural agents. For example, even the most comprehensive battery of mutagenicity (aka genotoxicity--damage to DNA) tests used today fails to spot chemicals that are already known mutagens (mutagenicity can lead to cancer; as it does for the chemicals in this test).[85]
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This simplistic ‘one gene, one protein’ theory immediately began to evolve towards the much more complex reality that is biology. A few short years (1958) after the discovery of the DNA double helix, the modulation of genes was discussed, terming it “epinucleic”.[86] Early on it was observed that many simpler organisms--amoebae and some amphibians--had massively more DNA than the more complex mammals. And as the bases of DNA strands of organisms began to be sequenced, the expected corelation between the number of *genes* and the complexity of organisms did not appear. E.g. the simple nematode worm C. elegans--fully developed, just 1,000 cells--has 19,000 protein-coding genes--close to the 15,000 genes of simple insects, yet complex humans have just 25,000 or so. On the other hand **and critically**, the amount of *nonprotein*-coding DNA <u>does</u> correlate with organism complexity.[87] Many *non-protein* signals to the cell and to the organism began to be discovered. Especially genes whose main job is to intract with an organism’s environment may be intrinsically designed to produce variations of their protein—in fact as many as 100 trillion variations for immune antibodies; or 10 trillion but more stable amino acid variations (at just 12 sites) of the protein legs of the hypordemic syringe-like phage viruses, whose tethers have to change as their bacteria victim’s outer membrane changes before they

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[84] S Freeland & L Hurst Apr. 2004 'Evolution Encoded', Scientific American, p. 84-91.

[85] G. Brambilla & Antonietta Martelli 2004 'Failure of the Standard Battery Of Short-Term Tests In Detecting Some Rodent And Human Genotoxic Carcinogens' [Review] Toxicology:196:1-2:1-19.

[86] J Lederberg Dec. 1958 ‘Genetic approaches to somatic cell variation: summary comment’ J Cell Physiol.:52(Supp 1):383-92; discussion 392-401.

[87] Mattick 2004.

can inject their RNA into the bacteria.[88]

SO, WHAT IS KNOWN ABOUT *HOW* EPIGENETICS REGULATES COMPLEXITY?

To begin with, almost all genes contain many introns--base sequences that are not part of the gene. Also there are a lot of exons outside of gene sequences—DNA that does not demarked as a gene. These are transcribed into RNA, as the gene is, but they are not translated into a protein. Rather, this RNA copied from introns and exons directs further expression of commands from the nucleus to the cell or organism. They appear to dominate much of development, reproduction and other functions of life.[89] In fact, this variability seems to explain how a very complex organism can have the same number of genes as a far less complex species.[90] This system in the more complex species is known as the "spliceosome" because it splices introns & exons out the DNA and attaches them to the RNA copy of a gene, thus creating many variations of a gene. Proteins and other genes too may be attached or play a role, for additional variation. Such complex regulation requires extra energy and creates vulnerability to some disease;[91] but it has certain efficencies, e.g. allowing the creation of different proteins from less than two genes-worth of DNA.[92]

The spliceosome is highly conserved, from yeast to humans. Though only recently discovered, it seems obvious that it is evolution's response to the challenge of controlling complexity. Humans, one of the most complex species, have the highest number of introns per gene of any known species. However, it was recently discovered that today's human introns are mostly very old, some predating the split of animals from plants (but still after complex eukaryotic cells arose).[93] Moreover that early intron complexity appers to have been more complex than today's introns are in complex organisms, implying that a certain amount of spliceosome rationalisation may have occurred over time. It is also known that even simple (early) prokaryotic were able to differentiate a bit as the organism developed,[94] implying a certain amount of complexity existed in cells without a dedicated nucleus.

In addition to the spliceosome system, many genes manage by other variations to create of more than one protein. Also, even if a gene turns out to code for just one protein, many proteins are already known to have two or more functions. In addition, many genes have variation in their sequence of bases (polymorphisms)--which modestly or seriously alter the function of the protein produced. Finally, discrete "genes" are a small part of DNA--around 99% of the DNA double-helix in human cells was originally misidentified as junk or 'non-sense DNA'.

Altogether, it's now evident that life receives far more signals from its chromosones than just one gene transcribed and translated into one protein to perform one task. Apart from direct transcription, DNA can be acted on in at least the following ways (such as by molecules and physical agents--foreign or endogenous):

- *Mutation*--direct physical damage to genes (tested for in today's chemical Q-RAs);
- *Modulation*--a direct but non-mutagenic interaction of a compound or force with a gene (as various hormones and their mimicks do) that changes the transcription of the gene; and
- *Epigenitic* instruction--i.e. events near the gene that thus **indirectly** influence gene expression, or that outright bypass gene instruction to the organism.[95]

To date, man has discovered that at least the following epigenetic (*non-gene*) elements in the chromosone that directly send commands to the organism: pseudogenes, anti-sense RNA, microRNA commands (including gene silencing, transcription and even organism development effects) and ribo-switches (some respond only to specific elements of the organism's present environment) and introns.[96]

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[88] SA McMahon 2005 Sep 18; [Epub; to appear in Oct. '05 issue] 'The C-type lectin fold as an evolutionary solution for massive sequence variation' Nat Struct Mol Biol.

[89] **E.g.:** JG Lee et al. 8 Nov. 2005 'Intronic Regulation of Matrixx metalloproteinase-2 revealved…' PNAS:102:16345-50.

[90] G. Ast April 2005 'The Alternative Genome' Sci. Amer.:58-65.

[91] Ast 2005.

[92] Ast 2005.

[93] S. Roy & W. Gilbert 8 Feb. 2005 'Complex Early Genes' PNAS:102:6:1986-91.

[94] Lee Kroos 22 Feb. '05 'Eukaryotic-like Signaling & Gene Regulation in a Prokaryote that Undergoes Multicellular Development' PNAS:8:2681-2.

[95] G. Felsenfeld & M. Groudine 23 Jan. 2003 'Controlling the Double Helix' Nature:421:448-53. This is a good overview of what's known about the role of the entire chromosone in transmitting instructions (including the epigenitic code), rather than focusing entirely on the small fraction of double-helix DNA strands that are genes. **Another** good primer (in two parts): W Gibbs 2003 'The Unseen Genome' Scientific American (Nov.: 'Gems Among the Junk' p. 47-53, Dec.: 'Beyond DNA' p. 107-13).

[96] Gibbs Nov. 2003.

One essential role of epigenitic instructions is to switch on or off the transcription of genes for extended periods of time.[97] Not only does is this 'gene silencing' critical to development and other periods, but silencing minimizes the amount of complex unwinding of the double helix strand from the spools it is stored on (overall called "chromatin", each spool is composed of proteins called histones): unziping the double-strand, transcribing the gene with RNA to make a protein, then reversing the process. Gene silencing's principal role in all of life can be seen in how perhaps all sexually-reproducing species use silencing to keep their females, who have two X chromosones, from transcribing no more of the X chromosone genes as males, who have one X and one Y chromosone. At least one if not all species has developed a system of propagating methylation silencing rapidly to all the genes of one X chromosone![98]

Even these " storage spools" are deeply involved in the complex signals that are life,[99] playing a role in gene transcription, cell replication and DNA repair.[100] At first, the original gene transcription theory was first modified such that the observed chemical modification on these histones led to loosening and tightening of a histone's grip on the DNA strands, allowing or suppressing gene expression; but now it is thought that such histone modifications serve as protein receptors, which then modify the availability of the DNA strand for gene expression.[101] An example, that also shows how subtle gene regulation is, is that families of genes--spread over thousands of base pairs--are regulated by long-range histone acetylation (a process that activates genes).[102] Different strategies of chromatin alteration (mostly to silence or activate genes) use many of the same proteins, in a coordinated strategy.[103] But now it turns out that histones are critical to far-downstream events--in the creation of muscles, being the first discovery.[104]

Far beyond its originally-discovered role as the template to transcribe a gene's sequence onto, it is now known that small strands of RNA (microRNA) are master regulators of the above gene expression/silencing activity. Nor is this activity simple on/off signals; but it includes (so far) silencing of mutant copies of genes and varying the degree of gene silencing. MicroRNA is also involved in directing gene transcription,[105] and it plays a role in the development of organisms, e.g. by combining with a protein to swich on 60 genes that convert stem cells to neurons.[106] In another example of the complexity of the original theoory, while it was originally thought that copying of bases into their RNA analogue proceeded along a gene in a simple smooth repetition; it was recently discovered that at least one of the enzymes involved--DNA polymerase--leaves and re-attaches to the DNA strand some 90 times in the course of a gene transcription (over some 15 minutes).[107] Even the original models of the steps of epigentic controls are turning out to be vastly simplified; e.g. how tRNA is processed.[108]

A possible example of epigenetic imprinting on the traditional theory of gene expression is the carcinogenicity and other chronic diseases induced by the super-potent estrogen diethyl stilbesterol (DES, a banned pregnancy drug). DES has been observed to permanently alter the downstream cellular functions of proteins (the products of genes) long after a single fleeting exposure to DES,[109] observations which may explain DES' known ability to cause cancer and reproductive effects in the grandsons & grandaughters of women who took DES during pregancy.[110]

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[97] Felsenfeld & Groudine 2003.

[98] A. Aleksyenko & M. Kuroda 20 February 2004 'Filling Gaps in Genome Organization' Science:303:1148-9.

[99] R. Waterland and R. Jirtle August 2003 'Transposable Elements: Targets for Early Nutritional Effects on Epigenetic Gene Regulation' Molecular and Cellular Biology:23:15:5293-5300. **Explained in**: Sandra Blakeslee 7 Oct. 2003 'A Pregnant Mother's Diet May Turn the Genes Around' New York Times, pg. 1 of 'Science Times' sec.

[100] Mone' et al. 2004.

[101] J. Travis 14 Feb. 2003 'Code Breakers' Science News:165:106-7.

[102] Weisong Zhou et al. 24 Feb. 2004 'Long-Range Histone Acetylation of he Ifng Gnee Is an Essential Feature of T-Cell Differentiation' Proc Nat Acad Sci:101:8:2440-5.

[103] T Owen-Hughes & M Bruno 16 Jan. 2004 'Breaking the Silence' Science:303:324-5.

[104] Hansol Lee et al. 11 June 2004 'Msx Cooperates w/ Histone H1b for Inhibition of Transcription and Myogenesis' Science:304:1675-8 (with a plain-English Perspective by Lisa Cirollo & K. Zaret 'A Linker Histone Restricts Muscle Development', p. 1607-8).

[105] E Hood Mar. 2004 'RNAi: What's all the noise about gene silencing?' Env. Health Perspectives:112:4:A225-9.

[106] J Travis 20 March 2004 'Brain Gain--Odd RNA Converts Stem Cells Into Neurons' Science News:165:179-80.

[107] Catherine Joyce 1 June 2004 'T4 Replication: what does 'processivity' really mean?' PNAS:101:22:8255-6.

[108]:E.M. Phizicky 2005 'Have tRNA, will travel' (Commentary) PNAS:102:11127-8.

[109] Thomas Weise 23 Oct. 2003, personal communication with Tony Tweedale. Dr. Weise is an endocrinologist at Xavier & Tulane U.'s in New Orleans.

[110] Carol Rice et al Oct. 2003 'Exposure Assessment for Endocrine Disruptors:..' Env Health Perspec:111:13:1683-90.

One (of several) mechanisms investigated to explain epigenetic effects on genes is the effect of alterations of the protective and gene-regulatory functions of **methylating** the base pairs of DNA, i.e. when they are coated with -CH3 (methyl) groups. It is largely methyl and acetyl groups that regulate the histone-spool's role in gene expression, described above. The end result of disruption of DNA methylation may again be the long-term disruption of cellular homeostasis.[111] In general, it appeats that unmethylated DNA is actively transcribed, while methylated DNA is silenced. Improper gene activation or inactivation via a change in methylation state obviously may cause disease; e.g. the often-observed silencing of tumor-suppresing genes. Proteins crucial to all stages of cell division, cancer metastasis and cell-suicide have been observed to be silenced by hyper-methylation.[112] In fact, cancer is well-known to be promoted by either methylation and de-methylation(!), in part depending on the stage of cancer development.[113] Methylation changes are ubiquitous in cancer.

Methylation is a strong component of gene activation/silencing (in combination with the transposon elements at the ends of genes, many which were acquired from viral DNA). E.g., methylation is a key control over how tightly the double-helix DNA strand is wound around the chromatin spools, which again may be a basic control on gene up-regulation (looser spooling) or down-regulation.[114] An example of the potent gene-regulatory role of methylation and transposons (both being epigenetic, not direct modulation mechanisms) is the recent finding that a *small* (3-fold) increase of these methyl groups in the diets of pregnant mice (as B vitamins) drastically changes the function of one of her offspring's genes, resulting in complete reversals of obesity and hair color (functions this gene was known to control), which of course may alter their future risk of obesity, diabetes and cancer.[115] It had long been known that nutritional changes in pregnant animals affects health throughout the life of the offspring, but no mechanism had been known. exposure to methylation-altering agents can have a huge and long-lasting affect, for example the apparant multi-generational effect on starved Dutch populations in World War II and malnourished populations after the U.S. Civil War and Great Depression. Yet too much methyl supplementation of nutrition (such as the unbiquitous fortification of flour with niacin (folic acid)) may be associated with autism and other diseases[116] (caution: as always, epidemiologic evidence may be confounded by variables such as the changes in macro-nutrients involved in this study).

Wholesale—radical, genome-wide--epigentic re-programming of genetic instructions occurs at some very early and critical stages after conception; obviously at least in part to silence some genes and activate others. Interestingly, female mammals (including human) use their X chromosone as a sink for methylating silencing proteins (and develop more slowly), while the Y male chromosone may act to activate more genes.[117] Obviously, this early re-programming adds even more complexity to biology; makes it more vulnerable to peturbations. For example, 'epigenetic drift': a study of identical twins (i.e. with identical genomes at conception) found that as their life unwound, each pair of twins' genomes began, and continued, to vary. This epigentic drift can have endogenous or exogenous causes, but the study found evidence for the latter—i.e. they found the more the twins' lifestyle varied (including living separately), the more epigenetic changes they had[118] Though the accompanying editorial cautions that it may be immune acquired responses that cause these genetic changes over a lifetime,[119] the paper discusses supporting evidence for these being direct epigenetic changes. First, the genetotic and phenotypic differences known to arise after animals have been cloned from a single genome. Second, contamination during assisted reproduction of sperm or eggs with methylating (gene silencing) molecules seems to cause defects and cancers.

Epigenetic control plays a role in at least some cancers, though one team has also shown that genetic changes and mutations remain critical to the genesis of cancer.[120] Supporting the above Waterland & Jirtle studies (evidence of increased risk of cancer from alteration of methylation) is that cancerous cells of many types consistently have

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[111] Rice et al. 2003.

[112] R. Ross & Barbara Spengler 18 Aug. 2004 'The Conundrum Posed By Cellular Heterogeneity in Human Neuroblastomas' J.NCI:96:16:1192-3.

[113] Yasuhiro Yamada et al. 20 Sept. 2005 'Opposing Effects of DNA Hypomethylation On Intenstinal & Liver Carcinogenesis' PNAS:102:38:13580-5.

[114] Waterland & Jirtle 2003.

[115] Waterland & Jirtle 2003.

[116] Waterland & Jirtle 2003.

[117] Marnie E Blewitt et al. 24 May 2005 'An N-ethyl-N-niotrosouera Screen for Genes Involved in Variegation In the Mouse' PNAS:102:21:7629-34.

[118] MF Fraga et al. 26 July 2005 'Epigenetic Differences Arise During the Lifetime of Monozygotic Twins' PNAS:102:30:10604-9.

[119] GM Martin 2005 26 July 2005 'Epigenetic Drift In Aging Twins' PNAS:102:30:10413-4.

[120] Christen Brownlee 'Biography of Rudolf Jaenisch' PNAS:101:39:13,982-4.

chromosones with altered methylation patterns.[121] Increased methylation of in cells of people eating known cancer-inhibiting nutrients has been observed.[122] The most universal tumor suprressor protein, p53, is in part regulated by methylation/demethylation (rather than its gene/s being so regulated).[123] Multiple cancer-promoting genes that are normally suppressed in both mice and humans can be activated by non-coding RNA retro-elements, with some evidence that that action indeed leads to cancer.[124]

In general, even minute and *fleeting* methylation alterations during the critical period of development may cause long-term downstream alterations in organism health. Additional evidence already associates methylation alteration with tumors[125] as well as the neurodegenerative disease Huntington's. In utero arsenic (still used as a pesticide) exposure is associated with many types of cancers, often at low doses--one mechanism seems to be that it prevents the methylation (silencing) of the estrogen receptor-alpha gene, which is then over-produced.[126] All this (and much more) from a field that is just getting started. In general, even minute and *fleeting* epigentic changes, such as in methylation, during the critical period of development, may cause long-term downstream alterations in organism health.

Furthermore, RNA is known to be subtly controlled by low concentrations of small molecules that bind to it and change its shape;[127] a "cooperative binding" process that originally was thought to exist to regulate proteins only, but now is known to regulate gene expression as well (via RNA control).

Moreover, proteins too, not just genes, are silenced or otherwise regulated—about 30% of proteins are phosphorylated at any time. While it had been assumed that this either switched the protein's signal on or off, it is becoming evident that protein activitycan increase gradually yet exponentially as its degree of phosphorylation decreases.[128]

Finally (in this third proof that 'the dose does *not* make the poison'), this complexity of genetic controls is--as always--being blindly ignored by arrogant scientists who, having learned *just a little bit* about these systems, began to tinker with them, thinking nobly how they will save humanity from a dreadful disease. But--also as usual--the response of the sytems is to display their complexity: i.e., the unntended consequence. "Oops" (not that some of these arrogant humans ever take the lesson to heart!). E.g., much tinkering has begun to use the short control fragments called RNA interference (RNAi--either natural or engineered), which often silences the expression of genes, as theraputic agents ("...RNAi, which was hailed for its 'laser-like specificity..."). Just as rapidly, nature has shown these arrogant idiots that RNAi's action is far from limitid to a single gene, for example they can activate the production of the immune response proteins called interferons.[129] Although the propensity of a single RNAi to cause the production of proteins associated with all or several of genes is not yet known, it is known that individual RNAi activate many (e.g. dozens) of genes, when such activity is tested in microarrays (which are simultaneous tests of the activiationof hundreds of genes).[130] A recent proposal to start a national epigenome project similar to the massive human genome sequence project just completed has been re-named as a proteonomics project in acknowledgement that such a survey of epigenetics will vary by cell type (unlike the human genome project). "The system is both more complicated and more specific than we previously thought."[131] Duh. Another clue that medical engineers are playing with fire comes from the discovery of RNAi(nterferance)--it was accidentally unearhted when researchers playing with RNA antisense (the opposite image of DNA that nature uses, thus

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[121] J Herman & S. Baylin 20 Nov. 2003 'Gene Silencing in Cancer In Association with Promoter Hypermethylation' NEJM:349:21:2042-54.

[122] Waterland & Jirtle 2003.

[123] S. Chulkov et al. 18 Nov. 2004 'Regulation of p53 Activity Through Lysine Methylation' Nature:432:353-9.

[124] Xu Song et al. 2005 'Roles of PSF protein and VL30 RNA in reversible gene regulation' PNAS:102:12189-93 [also: A. Deissereoth 30 Aug '05 'Normal and pathological functions of mammalian retroelements' (Coomente)PNAS:12292-3].

[125] Mitsiades CS et al. 2004 Jan 13 'Transcriptional signature of histone deacetylase inhibition in multiple myeloma: biological and clinical implications' Proc Natl Acad Sci U S A.:101(2):540-5 **and** Mitsiades N et al. 2003 May 15 'Molecular sequelae of histone deacetylase inhibition in human malignant B cells' Blood:101(10):4055-62.

[126] M. Waalkes et al. 17 Mar '04 'Estrogen Signaling in Livers of Male Mice With Hepatocellular Carcinoma Induced by Exposure to Arsenic in utero' JNCI:96:6:466-74.

[127] M. Fanulok 2004.

[128] J Gunawardena 11 Oct. '05 'Multisite Protein Phosphorylation Makes a Good Threshold But a Poor Switch' PNAS:105:41:14617-22.

[129] Jennifer Couzin 12 Nov. 2004 'RNAi Shows Cracks In Its Armor' Science:306:1124-5.

[130] Couzin 2004.

[131] D. Gius of the NCI, quoted in R. Tuma 17 Nov 2004 'Silencing the Critics: new studies move closer to answering epigenetics quetions' JNCI:96:22:1652-3.

rudimentaly stopping gene transcription) achieved their goal in their controls, not in their dependent variable![132]

Methylation and other gene silencings play a crucial role when cells simply replicate (mitosis--cloning), whether it's a differentiated or a stem cell. Gene silecing and certainly other epienetic controls are also crucially involved in the even more comlpex process of reproduction (meiosis--sex), where the chromosone is first replicated, then divided in half randomly for a seed cells' (sperm or egg) chromosone. Astonishingly in women this especially complex division process, begun in puberty, is not completed until conception--up to 40 years later (an incredible length to maintain viability, given the delicacy of cells)!

These ephemeral vital forces of replication and reproduction also are being brutally assaulted by greedy human biological engineers, who dream of wiping out disease and of building perfect humans. Fortunately, most serious biologists believe that gene silencing and other epigenetic controls constitute a barrier of complexity that these idiots will never overcome.[133] These replication and reproduction processes are stupendously complex and subtle; more so than the regular activities of cell life (where many generated molecular signals interact). These engineers have almost no idea how to control what happens when they crassly stimulate or alter these replication and reproductive processes. Their techniques seem to come from the 19th Century: blindly inserting an alteration into chromosones with almost no idea where it'll end up, therfore no idea what it'll do in addition to what it was designed to do; or in the case of imitating cloning and/or sex, literally applying electricity to jump start the cell replication process- with no (zero) idea of what else the electrical jolt is doing!

In sum, all chromosonal signals are vulnerable to upset; not just those of genes; as chemical Q-RA assumes.
Finally, as discussed above, the ***timing*** of exposure is one factor that can make the 'dose makes the poison' rule useless. An example of toxin timing that involves **epigenetics** is that (in at least four published studies) various chemicals in controlled experiments damage chromosones and/or DNA and lead to birth defects, but apparantly by epigenetic mechanisms.[134]
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Given all this evidence countering the 'one gene, one protein' theory, and that thousands of nonprotein-coding regions of vertebrate DNA have been conserved over millions of years of mutations, it may be better to conceptualize the role of proteins as: "islands of..information in a sea of [RNA] regulatory information."![135]

In summary, that ‘the dose makes the poison’ is a faulty, fundamental premise that has long defined classical toxicology and the protocols for chronic toxicity tests, which don’t test the dose levels we experience. Today's molecular biology has exposed the error of the outdated science of classic toxicology and the Q-RA methodology it engendered, showing that low dose effects and non-linear responses are common in biology.

PROBLEM 2: ARBITRARY SAFETY FACTORS *Non-cancer* chronic toxicity tests also apply the following “safety factors" to lower the NOAEL (i.e. to add a margin of safety):

- 10-fold to account for inter-species variability,
- 10-fold to account for intra-species variability (i.e., inter-individual),
- (sometimes)10-fold to account for toxcicology data gaps or poor quality data.

This reduction of the alleged NOAEL by up to 1,000-fold (typically it's 100-fold) results in the final, supposedly safe (non-cancer) dose used to regulate the chemical. It is another mockery of the scientific method and of logic: how can you quantify something when you can’t even approximate its numerical value? It's another inanity of chemical Q-RA, so if hard numbers are going to be used, Q-RAs should at least disclose that their safety factors are nothing more than a blind guess. On the other hand, cancer tests do not multiply their estimated safe dose by any safety factor because they theoretically test down to the lowest dose and because the test protocol is regarded as producing reliable data (however, they still assumes a simplistic linear dose-response relation).

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[132] M. Stevenson 21 Oct '04 ;Theraputic Potential for RNA Interference' NEJM:351:17:1771-7.

[133] R Jaensich 30 Dec. 2004 'Human Cloning--the science & ethics of nuclear transplantation' NEJM:351:27:278792.

[134] R. Chapin et al. Jan. 2004 'Off to a Good Start: the Influence of Pre- and Periconceptional Exposures...On Children's Health' Env. Health Perspectives:112:1:69-78 (discusses and cites four studies proving this, in its section titled: Preimplantation & Early Gestational Exposures).

[135] Mattick 2004.

Species differences is one more neglected field of study in toxicology, but major differences in response to dosing have been noted because chronic toxicity tests use two species. Occasionally, given time, chronic tests with different species are performed by other investigators, also leading to findings of species differences. Inter-individual (i.e. int*ra*-species) variation, both among humans and test animals, are widely said to be very significant for at least some responses; though again this is a poorly studied field. Finally, how can lowering the allowable dose by a few-fold make up for poor test protocols that fail to obtain the requisite knowledge needed to evaluate what they are supposed to evaluate? Pretending to quantify the unquantifiable is precisely what generates great scorn for Q-RA, and why it is not a branch of any natural science. What size "safety factor" is required to overcome the faulty assumption that low doses cannot ever cause an effect?

Recent advances in biology have touched-off an explosion that's closing this data gap's independent toxicology literature. Somebody estimated that *each cell* (each human has on average about 100 trillion cells)undergoes about 400,000 reactions *per second*. Proteins perform almost all of biology; humans have a few tens of thousand different ones. To maintain their many roles, some 33,000 proteins are destroyed per second in every cell, resulting in some 1.67 million fragments to further process every second in every cell.[136] About 100 million potassium ions *per second* cross a cell membrane that simulataneously bars other ions from passing into or out of a typical cell.[137] The human genome project has already established that each of our somatic (non-reproductive (germ)) cells has several million single nucleotide polymorphisms (SNP)--a variation in the DNA bases that make up a gene; or one SNP in about every thousand base pairs. About half a million of these SMPs may be significant, as they are found within genes (sequences of base pairs along the chromosones), and thus will code for the creation of a different protein than the gene normally creates. For example, locally, SNP variations in two genes that make proteins involved in detoxification means the cells of some Libby, MT residents are much more susceptible to the tremolite asbestos that the whole town is bathed in.[138] There are already at least hundreds of further examples, such as: typical body burdens of lead have very increased renal toxicity on people with a common gene variant.[139] Finally, test animals are always bred to be uniform (to aid the reliability of results). But this genetic uniformity significantly understates inter-individual variability of response ot the toxic agent being tested.

In sum, these three safety factors are obviously blind guesses; and most of the time they are likely inadequte ones. Identical logic applies to a fourth 10-fold safety factor that is just begining to be applied to "account for" the additional vulnerabilities of developing organisms.

In addition, pesticide registration now requires an additional 10x safety factor when evaluating children's risk, there is data showing children's risk is less; and US EPA's cancer RA Guidelines require that infants be considered 10x more vulnerable to age two (and 3x more vulnerable from age 2 to 15) than adults. Yet, for mutagenic carcinogens at least, it was recently shown that these safety factors tend to be inadequate: when dosed from birth to weaning, the central cancer response in test animals was 5 to 60 fold more sensitive than the adult animals; this held at the various dose levels used in the animal tests (9 carcinogens and four types of radiation).[140]

PROBLEM 3: *'TIMING*--NOT JUST DOSE--MAKES THE POISON': VULNERABLE DEVELOPMENT NOT TESTED

Instead of the dose determing the degree of harm, the timing of the dose, even an infenitesmal one, may cause harm. The golden rule of development is: a mis-timed or altered signal during adulthood typically causes transient ill effects, but during development it will cause permanent disruption.[141] Development could not be more important or vulnerable--for all organisms it is orders of magnitude more vulnerable to insult and upset than adulthood is.

We have generally emphasized that the timing of exposures may be as important as the dose; but during development

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[136] JW Yewdell 28 June 2005 'Commentary) 'Immonoproteosomes: regulating the regulator' PNAS:102:26:9089-90.

[137] Science News 9 March 2002, p. 152.

[138] Center for Env. Health Sciences (CEHS), School of Pharmacy, U. MT 2003; presented at the annual meeting of the MT Academy of Sciences, 26 April 2003, University of MT.

[139] Ming-Tsang Wu et al 'A [(ALAD)] Polymorphism May Modify the Relationship of Low-Level Exposure to Uricimia and Renal Function' Env. Health Perspectives 111:3:335-340.

[140] D. Hattis et al. Aug. 2004 'Age-Related Differences In Susceptibility to Carcinogenesis.: a quantitative analysis of empirical animal bioassay data' Env. Health Perspec.:112:11:1152-8.

[141] John MacLachlan 2003 'E.Hormone 2003' Tulane U. New Orleans, LA; Session 3: Developmental Patterning.

timing appears to be more critical. Unbelievably complex and critical cell differentiation is rampant (in humans from conception to ~20 years), evolving at least hundreds of radically different cell types from one. The healthy 20 to 60 year-old human is in relative biochemical stasis, literally closer to death than she is to her development. During development, the genes in most cells are cooperating to furiously produce the tens of thousands of proteins that perform the intricate tasks that take the organism from a mass of a few undifferentiated cells to its final shape. In short, every organism's potential depends on its development.

Despite such obvious complexity and criticality, chronic toxicity testing almost never begins before the end of the test animal's adolescence![142]

Moreover almost no chronic toxicity test lasts until old age so that the total effects of a toxin may be seen (chronic tests typically kill the test animals in the second half of middle age, both to save money and to allow for any possible chronic effects to develop). **The Ramazzini Institute in Italy is the only large tester of chronic toxicty which we know of that lets its dosed test animals live until they die; *tellingly, they often find chronic disease where others do not*!**

A related problem/fraud is that although to mimick typical exposure dosing should occur for most of the test-animal's lifetime, frequently dosing is shorter (e.g. 6 months or a year--in mammalian species that may be less than half the natural lifetime),[143] presumably mainly to again save expense in the care of test animals, but also to avoid confounding by the diseases caused by aging.

Of the end-points examined by chronic toxicity tests, no effects are assessed throughout development. The only exception is the developmental test--for birth defects, and some reproductive effects. These tests look at a fetus' development after dosing the mother; and sometimes the newborn is examined to some point in its life, occassionally up to its reproductive period. Typically though, only the partially-developed fetus is examined, even though different organs develop at different times, both pre and post-natal.[144] One clear experiment, cited in Problem #1 above[145]--finding that regulatory-required tests failed to find known neurotoxicity at levels a thousand-fold and more lower--ascribed that failure to not testing during development (which for these chemicals was already known to be neurotoxic to these much lower doses).

After many years, the U.S. EPA has finished revising its Guidelines for Carcinogenicty risk assessment; requiring risk be estimated using the total 'weight of the evidence' approach, in place of the narrower guidelines. Additionally EPA now assumes that exposure in infancy is 10 times more carnigoneic, and in childhood 3 times more, than during adulhoos. A review later validated the accuracy of these guesses: young lab animals were in fact about ten times more vulnerable to mutagenic carcinogens than animals dosed during late adolescencse to adulthood, and were 2.8 times more vulnerable to carcinogens acting by other mechanisms. What little direct human data exists (radiation and the estrogenic pregnancy drug, DES)also supported such early vulnerablity.[146] Given their far higher rate of biologic activity, it is unsuprising that infants are this vulnerable.

Despite this apparent confirmation of the protectiveness of these new cancer safety factors for infants and children, as just discussed in 'Problem #2 above, they may still be inadequate. The introduction to that paper (Hattis et al. 2004) identifies most of the infant exposure and metabolism factors that the EPA Guideline ignore, such as transplacental and lactational exposure. In the continuation of this validation testing that the EPA is supporting (rodent tests of actual cancer potency at different age of exposure), rodent lifetime cancer risk estimates that were 35%-55% higher than even this new Guideline allows for--i.e. the average increase in potency for exposures that include early life were 2.8 and 3.5 times more than the average potency for adult-only exposure (at least for the nine mutagenic carcinogens tested).[147]

That a simple, degradable and co-evolved with molecule as ethanol is deadly (in vitro, at doses experienced by fetuses in fetal alcohol syndrome) to the maturation of neurons only during developmental windows[148] does not bode well for the developmental safety of more persistent foreign molecules!

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[142] Dr. John Yargo, Yale U., videotaped presentation at 1997-1998 NCAMP conference; and EPA/OPPTS 1998.

[143] EPA/OPPTS 1998.

[144] Sandra Steingraber c. 2002 '*Having Faith*' (publisher unkown) has good examples of developmental vulnerability.

[145] Rice et al. 1996.

[146] H Barton et al. Sept. 2005 'Assessing Susceptibility From Early-Life Exposure to Carcinogens' EHP:113:9:1125-33.

[147] D Hattis et al. Apr. 2004 'Age-Related Differences in Susceptibility to Carcinogenesis. II. approaches for application and uncertainty anlysis for indiv. Genetically acting carcinogens' Env. Health Persp.:113:4:509-16.

[148] T. Lindsey & S Clarke Jan. 2004 'Ethanol Withdrawl Influences Survival & Morphology of Developing Rat Hippocampal Neurons in vitro' Alcohol Clin Exp Res.:28:1:285-92.

In fact, many hundreds of studies on developmental toxicity are now independently published every year. One example: the insecticide chlopyrifos ('Dursban', the latest of industry's chlorinated persistent termiticides to be banned for their incredible toxicity) has strong and different toxic effects on each of several of the key proteins controling the development of the three types of glial cells that make up much of the brain, as each develops in turn. There were also major male/female differences.[149] Another study carefully compared the effects of one dose of cadmium at different life stages: the younger the rodent, the more vulnerable its cells and tissues were.[150] One published herbicides example is that 2,4-D promoted pre-implanted cancers in rats when dosed near birth, but not when the exposure occured later in development.[151]

Low-dose developmental toxicity is now so widespread that one issue of a toxicology journal published two such reports from the same cohort (Italians at the accidental dioxin release in Seveso), by completely independent teams, looking at different endpoints. Such coincidencces happen regularly, but this instance was a bit notable, as both found that the timing of this dioxin exposure was the critical variable. In one, the failure to find an association of dioxin levels with early puberty (menstruation) was abscribed to the exposure occuring after birth in the women they studied (because that's how dioxin consistently affects test animals).[152] In the other, a variety of tooth aberrations (a development that is highly programmed, and it extends from 4 weeks after conception to ~20 years of age!) but not others were strongly associated with dioxin exposure.[153] In a dramatic example of dose timing, a genetic predisposition to uterine fibroid tumors was fulfilled by dosing mice with the potent estrogen DES shortly after birth; causing almost all to develop these tumors, while none of the genetically-safe mice got the tumors even after being dosed with DES. [154] Obviously, estrogenic chemicials at this early stage can re-program cells for later uncontrolled replication (cancer)--possibly by changing which genes are silenced and which are expressed (see our above discussion of epigenetics in Problem #1). The authors conclude that estrogen-responsive--even other--cancers may be affected by critical early toxic exposures.

Finally, a suite of male reproductive tract disorders which in humans has been exploding in incidence (see our following discussion)--low sperm counts, malformed uretheras, undescended testicles, and testicular cancer--may be controlled by in-utero exposure to hormone disrupting chemicals. The 'testicular dysgenesis syndrome' hypohesizes that in-utero exposure to hormone disruption re-programs the master Leydig cells of the testicles, resulting in these diseases in adulthood.[155] Recently prostate cancer (an adult disease and sensitive to estrogen disruption) was also shown to be influenced by near-birth estrogen imprinting.[156]

PROBLEM 4: FEW HEALTH ENDPOINTS EXAMINED Chemical Q-RA fails to look at many health effects, and fails to look thoroughly, when it declares the overall risk of a chemical. Chronic toxicity tests visually examine the tissues of the major systems and organs, and require preservation of tissue slides for potential microscopic examination. But these U.S. EPA/OECD country (Organization for Economic & Cooperative Development) chronic toxicity test guidelines (including for pesticide regulation) do not require that microscopic examination of any organ or tissue type be done, much less require comprehensive biochemical analysis (which is, also, a still-developing field).[157] These currently-used federal protocols were established before biochemistry and molecular biology, with their advanced tools, took their central place in biology.

According to these test guidelines, some organs need not receive even this crude visual microscopic examination. Also, important sub-tissues in organs (all of which are made up of more than one cell type) are missed, because there is no

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149 Stephanie Garcia et al. 2003 'Developmental Neurotoxicity Elicited by Prenatal or Postnatal Chlopyrifos Exposure: effects on neurospecific proteins indicate changing vulnerability' Env. Health Perspectives:111:3:297-303

150 M. Johnson et al. 2003 'Cadmium Mimics the Effects of Estrogen In the Uterus & Mammary Gland' Nature Medicine:9:1081-4.

151 A Parfieniuk et al. 1993 May 3-10 'Some parameters of Guerin cancer growth after exposure to Pielik (sodium salt of 2,4-dichlorophenoxyacetate)]' Pol Tyg Lek:48:414-6 **and** M. Sulik et al. 1996 'The effect of a herbicide--sodium salt of 2,4-dichlorophenoxyacetic acid on guerin carcinoma' Rocz Akad Med Bialymst:41:347-62.

152 Marcella Warner et al. Sept. 2004 'Serum Dioxin Concentrations and Age at Menarche' Env. Health Perspectives:112:13:1289-92.

153 Satu Alaluusua et al. Sept 2004 'Developmental Dental ABerrations After the Dioxin Accident in Seveso' Env. Health Perspectives:112:13:1313-8.

154 Jennifer Cook et al. 'Interaction Between Genetic Susceptibility & Early-Life Env. Exposure Determines Tumor Suppresor-Gene Penetrence' PNAS:102:24:8644-9.

155 R. Sharpe & N Skakkebaek 29 May 1993 'Hypothesis—Are oestrogens involved in falling sperm counts and disorders of the repoductive tract?' Lancet:341:1392-5.

156 O. Soder 1 Feb. 2005 'Perinatal Imprinting by Estrogen and Adult Prostate Disease' PNAS:102:5:1269-70.

157 EPA/OPPTS 1998, p.10-11.

specification to examine or to preserve each type of cell. The thousands of distinct biochemical functions of mammalian biology are not evaluated, because only a few are specified or recommended to be examined. Also, secondary but critical systems such as cognition, behavior, fertility, disease resistance, etc. are ignored (other than very gross, visually obvious effects noted during the tests). Male reproduction, chronic neurotoxicity, immune alteration and hormone function (the latter, critical to hundreds of functions) are some of the major functions not examined.

As to the route of exposure, dermal and inhalation exposures are seldom tested; typically only exposure by ingestion (food or water) is.[158] Thus there is usually no testing of additional or different injuries that may result from different metabolism or accumulation due to the different route of entry. In fact, much of what goes from the intestines to the blood is attached to lipoproteins that travel first to the liver; where toxins may have a greater chance of being excreted from the organism. That might make pther routes of entry of toxins into the blood far more dangerous, yet not evaluated by chemical RA!

Similar reality-checks on chemical Q-RA are featured in the findings of US EPA's Office of Pesticide Program's Scientific Advisory Panel (SAP) when reviewing the adequacy of EPA's new 10x child safety factor for pesticide risks. As pesticide industry as well as legitimate scientists are on the SAP, they naturally did not reach consensus that the standard tests are inadquate to determine chronic toxicity with. But many SAP members made it obvious that these tests are outdated, use insensitive indicators of toxicity, and ignore the time of exposure during development; and they advocated the consideration of human epidemiology studies--not animal experiments alone.[159] It is so obvious that the tens of thousands of biochemical systems of biology are inter-dependant in a complex organism, that it is absurd to assume that a chemical can only affect the biochemical endpoint being tested, as chemical Q-RA does.[160]

As late as year 2000, the USA's basic chemical safety law, the Toxic Substances Control Act (TSCA), had no regulation requiring chemcials be tested for reproductive/developmental and developmental neurotoxicity (now found at 40 CFR pt. 799). Even for the 3,000 most widely used chemicals (HPV, High Production Volume), the US EPA failed to require tests of all but four chronic and two acute exposure endpoints (health effects).[161] So even for these most dangerous exposures, only a handful of health endpoints and related tests are required, compared to the dozens of such tests in the draft E.U. REACh testing program.[162]

Genetic alterations may affect succeding generations not exposed to the causative agent. This is why reproductive developmental and teratogen test protocols have looked for effects in the offspring of a dosed parent. Once in a while these and other tests look for effects in the third generation, or for effects when only the father was exposed (where alterations of sperm are the only direct connection to offspring—for example some halogenated fumigant/fungicides). The reproductive effects, including cancer, to the grandchildren of women who took the pregancy drug diethyl stilbesterol (DES, a potent estrogen) are a famous example. Now that epigenetic alterations are begining to be invstigated (see above section), more such 'transgenerational toxicity' is being found. The pesticides methoxychlor and vinclozolin caused changes in epigenetic control of genes of male rodents through the 4th generation.[163] In humans, grandmother and mother's smoking was associated with asthma in the children/grandchildren, putatively through some genetic mechanism, not by direct smoke exposure).[164]

In sum, thousands of potential injuries are missed by these gross examinations--they are not much better than useless (cancers are easier to detect visually and microscopically, if they've been developing long enough; thus a carcinogenicity test is more reliable on this score.) Visual and/or microscopic examination are an absurd basis on which to claim a "no effect" dose, but it is surprising to see how many of these non-cancer chronic toxicity tests date from the 1970s and

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[158] Pesticide registration often (not always) requires some tests of these exposures, but most are acute tests (see our critique of pesticide registration below).

[159] FIFRA Scientific Advisory Panel (SAP) March 1998 Meeting Final Report, avail. http://epa.gov/scipoly/sap/1998/march/fqpa_10x.htm; accessed 13 Aug. 2004 (see Q. 1 of the 'Questions to the SAP' section).

[160] A good example of this inter-dependance of our biochemical systems is "metabolic syndrome" (endemic in Western society); where cardiovascular disease, diabetes and possibly some cancers interact, mediated in signifcant part by activation of the immune system (inflamation).

[161] Physicians Cmtee. for Responsible Medicine (accessed March 2003) 'The HPV Annimal Test Battery', http://www.pcrm.org/issues/pdfs/hpvappA.pdf .

[162] J Guth etal. July 2005 'The Louisville Charter Background Paper #5: Require Comprehensive Safety Data for All Chemicals', http://www.louisvillecharter.org/paper.safetydata.shtml (see 2 pg comparison table at end).

[163] M. D. Anway 3 June 2005 'Epigenetic Transgenerational Actions of Endocrine Disruptors and Male Fertility' Science: 308:5727:1466-1469.

[164] Yu-Fen Li et al. Apr. 2005 'Maternal & Grandmaternal Smoking Patterns Are Associated With Early Childhood Asthma' Chest:127:1232-41.

earlier are still cited by Q-RAs today.[165] It is again expense that drives this problem (as it does the 'unrealistic doses' problem #1, above). The roughly $20,000 to 100,000 per chronic effects test is spent, in part, on the care and dosing of hundreds of animals over most of their lives. But the other cost of the tests--the examination of the animals after dosing is done--is what causes the large variation in the cost, and it's this large variation in cost that varies with the extent and quality of the analysis.

PROBLEM 5: SINGLE-CHEMICAL TESTING ONLY Of the approximately 100,000 chemicals that have entered commerce to date,[166] humans and the biosphere are exposed simultaneously to thousands of synthetic and processed chemicals. Pesticide formulations are an example of our real-world exposure to mixtures--whether persistent, or non-persistent but ubiquitous. Even though the safety review required to register a pesticide for use is endlessly (but erronously--see our critique) cited as an example of thorough safety testing, this testing is only done on the ingredient claimed to have pesticidal activity, called the "active ingredient" (a.i.). Pesticide formulations are a typical example of Q-RA's failure to evaluate our real exposure to mixtures. Of one of our examples of low-dose effects above, the M. Cavieres et al. 2002 low-dose Trimec (a triple a.i. herbicide formulation) study, one of the authors, Warren Porter, expressed this failure of Q-RA well when he said:

> "You're talking about putting a lot of very reactive chemicals together in a mix, and storing it at room temperature . We have no idea what kind of reactions might be going on once these active ingredient are formulated into products."[167]

Such studies indicate caution for pesticide formulations alone--never mind that pesticide use is added to the soup of tens of thousands of chemicals in the biosphere.

As we have established how subtle biology is, there should be no expectation that the classsic Q-RA system of testing chemicals singly can predict what happens in our real mixed exposure world. As of Oct. 2003, there were over 22 million existing and future chemicals registered with the Chemical Abstract Service (CAS), which attempts to assign a unique ID number to each. Of the 100,000 chemicals in commerce worldwide, as we show in Problem #6 below, chemical Q-RA fails to, even individually, evaluate more than a small minority--when Q-RA does enter the game, 95% of toxicology experiments are estimated to involve a single chemical.[168] Yet almost all the remaining 5% of studies simply study the effects of common mixtures of toxins, e.g. as in urban air, water chlorination byproducts, etc.; rather than attempt to determine the toxicity of the interactions of the components.

That is so because it is fundamentally impossible to test the effect of more than a few combinations--e.g. only the very largest use chemicals. The combinations of just 100 chemicals in groups of four (let alone in groups of two) would require 3.9 million full-blown tests![169]. It has been said that to test all unique combinations of the chemicals in commerce to date (about 100,000 chemicals; so the number of unique combinations has been said to be 2 to the 100,000 power, minus 100,001) would require more test animals than there are stars in the known universe--billions of galaxies, each with billions of stars. Even a short (13-wk.) animal toxicity test for all combinations of just 25 chemicals (far ewer than we have contimanting our bodies) requires 33 milion separate tests. Testing just 1% of chemicals in commerce (700 chemicals) would require 10E210 tests![170] All these calculations of course assume we just care about one health effect. No wonder chemical Q-RA recoils from such a task, despite its obvious pertinence!

A startling example of the problem that mixtures pose to chemical Q-RA involves the well-known carcinogenic and mutagenic (damage to DNA, which can lead to cancer, birth defects and other chronic disease) polycyclic aromatic hydrocarbons (PAHs)--complex but ubiquitous mixtures in almost all combustion emissions, their production enhanced under low-oxygen (smoldering) conditions--also called creosote. PAH toxicity had been thought to be well-enough studied for many Q-RA to use the mutagenicity and cancer potency of one very toxic PAH, benzo(a)pyrine (BaP), to characterize the cancer risk of all PAHs; sometimes even adjusting for the assumed lesser potency of other PAHs. Yet a

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[165] For example, see one of the Q-RA relied on by this FEIS, which we critique in a following section--the anonymous 'Risk Assessment for USFS Regions 1, ... and the BPA'. It has a thorough risk analysis (i.e. literature review) section, many of which papers date from the 1970's and earlier.

[166] R. Thomas et al. (Dec. 2002) 'Applications of Genomics to Toxicology Research' Env. Health Perspectives:110(Suppl. 6):919-23.

[167] E. Green 17 Sep. '02 'Study Links Weed-Killer to Reproductive Problems' Los Angeles Times.

[168] V. Feron et al. (Dec. 2002) 'International Issues on Human Health Effects of Exposure to Chemical Mixtures' Env. Health Perspectives:110(Suppl. 6):893-9.

[169] Peter Montague 17 Oct. 2002 Rachel's Env. & Health News #754 (available: http://www.rachel.org). That is perhaps before subtracting the repeating permutations.

[170] Tim Montague 23 June 2005 'Isn't It Time We Regulated Chemical?' Rachel's Env. & Health News #754 (http://www.rachel.org).

set of extensive experiments strongly indicate that BaP is not an accurate predictor of the mutagenicity or carcinogenicity of various PAH mixtures (the coal tar byproduct of synthetic gas plants). Various BaP and coal-tar mixures have very different cancer potencies, despite the assumption of every Q-Ra to the contrary.[171] That chemical Q-RA competely failed to estimate the risk of even a ubiquitous real-world exposure is a beautiful illustration of its incompetence, though not a suprise. In fact, there is additional literature showing contradictory results of the carcinogenicty and mutagenicity of various PAH components of combustion emissions, summarized in another paper presented at the same symposia on mixtures.[172] Also, all Q-RA for PAHs assume that very little is absorbed from the gut (for the ingestion pathway) due to their binding to organic matter in the colon, which also is assumed to inactivate their toxicity. Yet it was recently shown that these Q-RA lmay underestimate PAH toxicity by around 12-fold, because PAHs (soil-bound or not) still exhibited strong estrogenicty (one indicator of toxicity) after being digested and metabolized in the colon.[173] What's the overall lesson of this example of PAHs in Q-RA?: why are we so stupid as to assume that mixtures of many compounds will act uniformly, or that there wont be interactions?

Also, it has been shown that concentrations of different estrogens (including estrogenic pollutants) that do *not* elicit an estrogenic response alone, more than *double* the test system's response to estradiol (the bioactive form of estrogen in most animals, including humans) when combined in a real-world mixture.[174]

It is certainly true that antagonism--the toxic effect of one agent being cancelled by another--occurs; perhaps as often as synergism (a greater than additive effect) does. But far more important is that synnergy or antagonism are measurements of the effect of mixtures on one biochemical system only, whereas obviously essentially all our organs and biochemical systems are exposed to mixtures. Biochemical inter-actions are also a failure of individual chemical testing (as pointed out above) but these biochemical inter-actions seem obviously likely to be far more complex when caused by a mixture. Germany's MAK Commission, the equivalent of the USA's NIOSH (Ntl. Institute for Occupational Safety & Health), both which recommend putatively safe air levels of agents to their respective workplace regulatory agency, states this problem well:

> "A MAK value for a mixture of substance cannot be satisfactorally determined by simple calculations because the components of the mixture generally have different kinds of effects; ...Given the inadequacy of the currently available data, the comission decidedly refrains from calculating MAK values for mixtures."[175]

As with the other failures of Q-RA, the small independent literature that *has* developed on mixtures (since there is no requirement be test for it) seems to show hundreds, if not a few thousand examples; because we, without doing any particular literature search for it, have dozens of examples of synergy. At least two recent symposia in chemical mixture toxicology have been recently published.[176], [177] Without even a specific literature search, we know of a few dozens of mixture toxicity studies *in the very narrow chemical catogory of* ***herbicides, alone*** (most are examples of defininte synergy, because enough testing was done to see if effects were greater-than-additive):

> The above twice-mentioned study of Trimec, a three-herbicide formulation (failed pregnancies in rats, at environmentally relevant doses).[178]
>
> Chlorophenoxy herbicides (dicamba, 2,4-D and MCPP, a similar mix to Trimec) along with formulation ingredients naptha, naphthalene and nitroaniline (at concentrations below occupational limits) were drawn into a building's air intake; causing respiratory and neuorologic acute effects, but with at least one permanent respiratory injury.[179]

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[171] L. Goldstein et al. 1998 'Tumors and DNA Adducts in Mice Exposed to Benzo(a)pyrine and Coal Tars: implications for risk assessments' Env. Health Perspectives:106(Suppl. 6):1325-1333.

[172] S. Nesnow et al. 1998 'Lung Tumorigenic Interactions in Strain A/J Mice of Five Environmental Polycyclic Aromatic Hydrocarbons' Env. Health Perspectives:106(Suppl. 6):1337-1346.

[173] T. van de Wiele et al. Jan. '05 'Human Colon Microbiota Transform PAH to Estrogenic Metabolites' Env. Health Perspectives:113:1:6-10.

[174] Rajapaske et al. 2002 'Combining Xenoestrogens at Levels Below Individuals No-Observable Effect Concentrations Dramatically Enhances Steroid Hormone Action' Env. Health Perspectives 110:917-921.

[175] R. Bartsch et al. 1998 'Maximum Workplace Concentration Values and Carcinogenicity Classification for Mixtures' Env. Health Perspectives:106(Suppl. 6):1291-1293.

[176] W. Suk, K. Olden & Raymond Yang, eds. (Dec. 2002) 'Application of Technology to Chemical Mixture Research' Env. Health Perspectives:110:891-1036.

[177] Raymond Yang & W. Suk, eds. (Dec. 1998) 'Current Issues on Chemical Mixtures' Env. Health Perspectives:106(Suppl 6):1261-1448.

[178] Cavieres et al. 2002

[179] H. Zeliger Jan. 2003 'Toxic Effects of Chemical Mixtures' Arch Env Health:58:1:23-9 (see case #6).

A herbicide formulation poses a greater risk to human fertility than indicated in trials of its separate ingredients. In a study of wheat, sugar beet and potato farm workers, birth defects were doubled among children of crop workers that were conceived during months when the pesticide 2,4-D was sprayed (recently confirmed and elaborated in a new study by this team). They note that in toxicity tests for its registration, pure 2,4-D was used, while workers actually handle a blend of 2,4-D and added ingredients.[180]

Older women exposed to triazine herbicides and thiocarbamate insecticides had eight times the rate of spontaneous absorption as those exposed to triazines only.[181]

Atrazine and alachlor herbicides together are more acutely toxic to amphibian larvae than alone.[182]

Atrazine and possibly other pesticides synergize the infection of amphibian species such as frogs with the trematode worm. This parasite has been shown to be a direct cause of the frog limb deformities that are being observed so frequently in the field.[183] (atrazine itself may contribute to the amphibian decline, given its ability to disrupt their sexual development at environmental concentrations[184]). At environmentally relevant doses the immune system of frogs are severely depressed by the insecticides DDT, dieldrin and malathion--in the lab and in the field;[185] thus theoretically these insecticides at least make frogs very vulnerable to the trematode parasite, which is usually observed in the evident epidemic of frog limb malformations.

N-NitrosAtrazine (N-NAt) increases chromosone breakage in-vitro at just 0.1 ng/ml (roughly, 0.1 ppb); wheras nitrates, nitrite or atrazine alone required concentrations 1,000 to 10,000 times higher to do so. Even when admistered together they caused less chromosone damage than the N-NAt metabolite.[186]

Atrazine and metachlor (only when dosed together) delay metamorphosis of tadpoles to frogs by 10 days, at concentrations found in farm run-off.[187]

The same team recently tested a typical agricultural pesticide mix (the herbicides atrazine, metolachlor, alachlor, metylaxyl & one other, three insecticides and two fungicides). Alone they did not cause developmental abnormalities in frogs at 0.1 ppb (a low, env. relevant dose) but in creating a mix one by one (at the same doses), adverse effects appeared and amplified--basically in proportion with the number of added pesticides! The effects were slowed larval growth, development & metamorphosis; less immune response; and more production of stress hormones (which are known to slow growth).[188]

A mix of 2,4-D, glyphosate and triclopyr herbicides was toxic to larvae at levels lower than recommended for field applications.[189]

Picloram plus 2,4-D formulations (Tordon 202c) are a potent birth defect combination of a.i.[190]

While neither of these two a.i. alone damages the gill cells of catfish, in one of these Tordon formulations they

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[180] V. Garry et al. 1996 'Pesticide Appliers, Biocides and Birth Defects in Rural Minn.' Env. Health Perspectives 104:394-399. (Their **follow-up** study is Garry et al. 2002; below).

[181] Arbuckle et al. 2001 Env. Health Perspectives:109:851-7.

[182] GE Howe et al. 1998 'Effect of Chem. Synergy & Larval Stage on the Toxicity of Atrazine & Alachlor' EnvToxicol Chem:17:519-525.

[183] J. Kiesecker 2002 'Synergism Between Trematode Infection and Pesticide Exposure, a Link to Amphibian Deformities in Nature' Proceeding Ntl. Academy of Science:99:9900-9904. **See also** A. Balustein & P. Johnson Feb 2003 'Explaining Frog Deformities' Sci. American, p. 60-5.

[184] Hayes et al 2003.

[185] M. Gilberstson et al. 2003 'Immunisuppression in the Northern Leapord Frog (Rana pipiens) INduced by Pesticide Exposure' Environ Toxicol & Chemistry:22:1:101-10.

[186] L. Meisner et al 1993 'In-Vitro Effects of N-Nitrosatrazine On Chromosone Breakage' Arch. Env. Contam. & Tox.:24:108-112.

[187] Hayes et al 2002; **as reported in** J. Raloff 2 Nov. 2002 'More Frog Trouble: herbicides may emasculate wild males' Science News.

[188] T. Hayes et al. 2004, new data presented at symposium "Ecophysiology and Conservation: The Contribution of Endocrinology and Immunology" held during the Society for Integrative and Comparative Biology (SICB) meeting January 5-9, 2004, in New Orleans, Louisiana.

[189] Abdelghani et al. 1997 'Toxicol. Evaluation of Single & Chem. Mixtures..' Env. Toxicol. & Water Quality:12:237-43.

[190] P. Blakley et al. 1989--**3 papers**: Teratology:39-237-41 **and** 39:547-53; **and** J. Tox. & Env. Health:28:309-16.

do, an outright proof of synergy.[191]

A series of papers on similar formulation with the same two-a.i. (Tordon 75D)permanent testicular damage, often due to “inert” ingredients of the formulation, e.g. the detergents used; and severe depression of respiration in mitochondria for the mix but not the indiv. a.i., while a final study failed to elicit reproductive or teratogenic effects in the offspring of fathers dosed with Tordon 75D.[192]

A separate confirmation of the above mitochondria respiration toxicity, except using picloram with 2,4-D, also shows shows an interaction with complex I of the respiratory chain, and a partial collapse of the proton motive force of the mitochondrial inner membrane without affecting its elasticity.[193] Early studies of this 'Agent White' mix looked at egg-hatching success;[194] and a more recent study examined the acute aquatic toxicity of 2,4-D, triclopyr and glyphosate mixes.[195]

Combinations of the herbicides alachlor, atrazine and picloram had various synergistic effects.[196]

Various combinations of the herbicide atrazine, the insecticides aldicarb and carbamate, and nitrate (fertilizer) were synergistic at the concentrations they are typically found at in groundwater.[197]

In a series of published studies, atrazine potentiates the toxicity of orgnao-posphate insecticides to midge insects.[198]

Later exposures to any toxin are made more dangerous by 2,4-D’s de-activation of detoxifying liver enzymes.[199] Organophosphate insecticides are degraded by the same enzyme in some plants & crops, thus the metabolism of either may be slowed by the other; causing damage. Some OPs, at least, are known to be taken up more thoroughly.[200]

In protein deficient rats, the chlorinated herbicide diuron’s acute toxicity was significantly enhanced, and their sperm production was halted; while normally fed rats were not affected.[201]

The herbicide linuron and a mixture of 15 pesticides (mostly insectivdes and fungicides) commonly found in the Italian diet act as additive promoters of existing cancers in test animals, implying a risk at current population-wide

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[191] Gallagher & DiGuillo 1991 ‘Effects of 2,4-D and Picloram on Biotransformation, Peroxisomal...’ Toxicol. Letters:57:65-72.

[192] D. Oakes et al. 2002 ‘Testicular changes induced by chronic exposure to the herbicide formulation, Tordon 75D ® (2,4-dichlorophenoxyacetic acid and picloram) in rats’ Reproductive Toxicology:16 281–289; **and** D. Oakes & J. Pollak 2000 ‘The in vitro evaluation of the toxicities of three related herbicide formulations containing ester derivatives of 2,4,5-T and 2,4-D using sub-mitochondrial particles’ Toxicology 151:1–9; **and** D. Oakes & J. Pollak 1999 ‘Effects of a herbicide formulation, Tordon 75D ® and its individual components on the oxidative functions of mitochondria’ Toxicology:136, 41–52; **and** Oakes DJ, et al. 2002. A study of the potential for a herbicide formulation containing 2,4-d and picloram to cause male-mediated developmental toxicity in rats. Toxicol Sci 68:200-206.

[193] Pereira LF, Campello AP, Silveira O. 1994 Jan-1994 Feb 28. Effect of tordon 2,4-d 64/240 triethanolamine br on the energy metabolism of rat liver mitochondria. J Appl Toxicol 14:21-26.

[194] Somers J et al. 1974 Jan. Effect of external application of pesticides to the fertile egg on hatching success and early chick performance. **1.** Pre-incubation spraying with DDT and commerical mixtures of 2,4-D: picloram and 2,4-D: 2,4,5-T. Bull Environ Contam Toxicol 11:33-8. **2.** Commercial-herbicide mixtures of 2,4-D with picloram or 2,4,5-T using the pheasant. Bull Environ Contam Toxicol 11:339-42.

[195] Wan MT, Watts RG, Moul DJ. 1991 Sep. Acute toxicity to juvenile Pacific northwest salmonids of basacid blue NB755 and its mixture with formulated products of 2,4-D, glyphosate, and triclopyr. Bull Environ Contam Toxicol 47:471-8.

[196] Information Ventures Inc. 1999 ‘Review of the Literature in Herbicides...IV. Health Effects of Other Herbicides’ avail. at http://infoventures.com/e-hlth/ .

[197] W. Porter et al. 1999.

[198] Schuler LJ et al. 2005 Aug ‘Joint toxicity of triazine herbicides and organophosphate insecticides to the midge Chironomus tentans’ Arch Environ Contam Toxicol:49(2):173-7.

[199] Rachel Carson 1962 ‘Silent Spring’ Houghton Mifflin (Chapters 13-15).

[200] R. Hartzler 5/22/2000 'Interactions between ALS-herbicides & organophosphate insecticides' Integrated Crop Management is published by the Dpt. Entomology, Iowa State U. Ames, Iowa http://www.ipm.iastate.edu/ipm/icm/2000/5-22-2000/interaction.html (accessed early 2004).

[201] E. Boyd & V. Krupa 1970 ‘Protein Deficient Diet and Diuron Toxicity’ J. Food Chemistry:18:1104-7.

exposure levels.[202]

Several studies have now definitively shown that pesticides, including herbicides, act in concert with the trematode parasites to cause the widely-observed deformities in amphibians. Tadpole vulnerability to u.v. in sunlight may also contribute.[203]

When the estrogenicty of ten pesticides was added it was weaker than estradiol, the active form of estrogen, but in various combinations they had potent and synergistic estrogenicity.[204]

Two applicators were killed from the spill and inhalation of two of the herbicides mentioned for use in this FEIS, imazapyr and triclopyr--just 13 lbs. of the latter, an unstated amount of the former.[205]

In the temporal and geographic vicinity of organophosphate insecticides, the unbelievably potent ALS inhibiting herbicides (the sulfonureas and the imidazaloninones) are even more (synergistically) potent.[206]

Various amounts of pesticides alone do not kill tadpoles, but when a single predator (separated from the tadpoles by a net) is added, they die. Six frog species are so affected with the insecticide carbaryl (Sevin); and the same result for "a common herbicide" is about to be published.[207]

One research team has so far published at least **eight papers** studying the serious synergistic neurotoxicity of the herbicide paraquat with some fungicides (often used together in the real world), especially Parkinson's Disease.[208]

12 of the 13 pesticide active ingredients significantly arrested very early mammalian development--a period that EPA's developmental test for pesticides fails to cover (typical!), even though the doses were at EPA's Reference Dose for the pesticide--a level 100 times (typically) lower than any adverse non-cancer effect is supposed to happen at! Similarly, five of six mixtures (three or four of the 13 in each, including a common lawn care herbicide mix and pre & post-emergent herbicide mixes) significantly arrested development.[209]

Immune B-cell populations are decreased after exposure to propanil, 2,4-D, or the mixture containing propanil and 2,4-D. Exposure to the mixture had greater toxic effects than the individual herbicides on bone marrow pre-B and IgM(+) B-cell populations.[210]

Channel catfish exposed to a mixture of picloram and 2,4-D for 10 days displayed increased activities of hepatic ethoxyresorufin O-deethylase (EROD), decreased serum chloride concentrations and decreased liver/body weight ratios. These changes were not observed in fish exposed to either compound alone.[211]

In a prospective, controlled study of applicator exposures (insecticides, fungicides, herbicides) only the herbicide group showed significant endocrinologic differences from controls. In vitro genotoxicity examination compared four different commercially available surfactant mixtures with 12 different commercial herbicide products, including six different chlorophenoxy herbicides. Only one herbicide yielded a significant dose-response curve. All four adjuvants showed positive dose-response effects [apparantly only certain ranges of the dose curve were

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202 Pasquini R et al. Sep-1994 Oct 31. Assay of linuron and a pesticide mixture commonly found in the italian diet, for promoting activity in rat liver carcinogenesis. Pharmacology & Toxicology 75:170-176.

203 R. Blaustein, P. Johnson ‘Explaining Frog Deformities’ Feb. 2003 Sci. American, p. 60-5.

204 A. Soto et al. 1994 'The Pesticides Endosulfan, Toxaphene, and Dieldrin Have Estrogenic Effects on Human Estrogen-Sensitive Cells' Environmental Health Perspectives:102:380-383.

205 ATSDR 2002 ‘HSEES 1999-2000 Biennial Report’, Atlanta GA (see Appen. C).

206 R. Hartzler 2000.

207 Rebecca Renner Apr 2004 'Double Distress: pesticide kills frogs only if predators are around' Sci. American p. 32 (referencing R. Relyea in the Dec. 2003 Ecol. Applications; and soon-to-be-published for the herbicide experiments).

208 Deborah Cory-Schlecta, U. Rochester, is the team leader--search PubMed on her name for the abstracts.

209 Anne Greenlee et al. May 2004 'Low-Dose Agrochemicals and Lawn-Care Pesticides Induce Developmental Toxicity in Murine Preimplantation Implants' Env. Health Perspectives:112:6:703-9.

210 de la Rosa P, Barnett JB, Schafer R. 2003 Dec 26. Loss of pre-b and igm(+) b cells in the bone marrow after exposure to a mixture of herbicides. J Toxicol & Environmental Health 66:2299-2313.

211 Gallagher EP, Di Giulio RT. 1991 Jun. Effects of 2,4-dichlorophenoxyacetic acid and picloram on biotransformation, peroxisomal and serum enzyme activities in channel catfish (Ictalurus punctatus). Toxicol Lett 57:65-72.

examined]. These preliminary data suggest that adjuvants are not inert but are toxicologically active components added to herbicide mixtures. Whether adjuvant toxicant effects are additive or are independent of herbicide effects is poorly understood.[212]

The presence of single detergents and their mixtures increased promethrin herbicide effects by 10-13% on the testes invertebrates even in concentrations permitted in surface waters. The toxic effect of 2,4 D was potentiated by detergents in much higher concentrations, exceeding the permitted values.[213]

Food deprivation tended to decrease the sensitivity of rats to the effects of either of two 2,4-D formulations. The spectrum of neurobehavioral effects varied with the ester isomers.[214] Three formulations of 2,4-D were tested in rats for their ability to increase landing foot splay, a measure of ataxia. Results suggest that the 2,4-D-n-butyl ester metabolite n-butanol is responsible for the motor incoordination but not the depression of locomotor activity observed, demonstrating that different formulations of the same herbicide can produce differential behavioral effects.[215]

Yet another mixed-herbicide formulaton available for sale (2,4-D and propanil) was more toxic to immune system cells in vivo than was either herbicide alone, and treatment with a glucocorticoid receptor antagonist (propanil was previously known to increase corticosterone, leading to immune cell loss) did not protect against bone-marrow immune cell loss from exposure to the mix.[216]

Eight supposedly non-genotoxic chemicals were indeed genotoxic (mutagenic) under conditions of oxidative damage stress (which cells undergo all the time), including the herbicides 2,4-D and glyphosate.[217]

Glyphosate and the ingredients it is formulated with (in the product Roundup) interfere synergisticaly with cell division in vivo in sea urchin embryos.[218]

It is well established that fat soluble (lipophilic) chemicals are generally easily absorbed through the mucus membranes and other barriers of skin, lungs and the gut;[219] many of which are very fatty ["like dissolves in (is attracted to) like"--ultimately this is due to the neutral charges of lipophilic molecules such as fat or other high-carbon content molecules, versus the highly charged areas of water soluble (hydrophilic) molecules, including water]. This is relevant to mixtures because co-exposure of the mucus membranes to chemicals of differing solubility, such as found in many products and real-world exposures, causes the more of the water soluble (hydrophilic) chemicals to be absorbed into the body than would otherwise be the case--an example of co-solvency. In fact, many instances of health effects from such chemical mixtures have been ascribed to such co-solvency.[220] Most of these mixture effects were caused at doses below concentrations allowed by regulatory bodies. The co-solvency toxicity effect was conclusively shown in another study, where lipophilic chemicals synergized the toxicity of hydrophilic chemicals when both were applied to human mucus membranes. In fact, the more fat-soluble a chemical was, the greater the toxicicty of the water soluble co-chemical.[221] That water-soluble chemicals are more reactive than fat-soluble chemicals (due to the excess electrical charges that they carry) may play a role in the effects observed in these situations; because reactivity is also called oxidative damage, a

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[212] Garry VF et al. 1999. Herbicides and adjuvants: an evolving view. Toxicology & Industrial Health 15:159-167.

[213] Ranke-Rybicka B, Plachta J, Zycinski D. 1995. [Effect of water contamination with surface active substances and plant protecting agents on aquatic organisms]. Rocz Panstw Zakl Hig 46:175-81.

[214] Schulze GE. 1987 Sep-Oct. Formulation and food deprivation affects 2,4-D neurobehavioral toxicity in rats. Neurotoxicol Teratol 9:363-7.

[215] Schulze GE. 1988 Jan-Feb. 2,4-D-n-butyl ester (2,4-D ester) induced ataxia in rats: role for n-butanol formation. Neurotoxicol Teratol 10:81-4.

[216] P. De La Rosa et al. 2003 'Loss of pre-B and IgM(+) B cells in the bone marrow after exposure to a mixture of herbicides' J. Toxicol& Env. Health-Part A:66:2299-313.

[217] A. Lueken et al. 2004 Feb 28 'Synergistic DNA damage by oxidative stress (induced by H2O2) and nongenotoxic environmental chemicals in human fibroblasts' Toxicol Lett:147:35-43.

[218] J. Marc Mar. 2002 'Pesticide Roundup provokes cell division dysfunction at the level of CDK1/cyclin B activation' Chem Res Toxicol.:15(3):326-31.

[219] L. Alessio 1996 'Multiple Exposures to Solvents in the Workplace' Int Arch Occup Environ Health:69:1-4.

[220] Zeliger 2003.

[221] I. Witte et al. 1995 'Correlation of Synergistic Cytotoxic Effects of Env. Chemicals in Human Fibroblasts With Their Lipophilicity' Chemosphere:31:9:41-9.

common mechanism of damage to organisms.

PROBLEM 6: FAILURE TO TEST MOST CHEMICALS, *EVEN INDIVIDUALLY* Synthetic chemicals became intrinsic to society so rapidly following the German organic chemistry revolution that they were almost entirely introduced into use without testing individually (much less testing the real-world soup mixture the biosphere lives in). Pharmaceuticals are the only chemicals that in some countries must be shown to be safe before they can be sold (ironic, as they are chemicals meant to improve health!). Pesticides may be required (as in the USA) to undego safety testing before being sold (also ironic: the only category of non-therapeutic (e.g. cancer chemotherapy agents) chemicals *designed* to be poisons!), but such tests are at best a balancing of health and ecomomic costs and benefits (i.e it's C/BA, a bigger fraud than even chemical Q-RA; as mentioned in the Introduction). No other synthetic chemicals—not even personal care chemicals—require any sort of safety review before sale.

Finally, those regulatory systems descriptions are ideal. In reality, even the stringent pharmaceutical testing has been *completely* captured by the industry it is supposed to regulate (see Problem # 8 below). Yet even conceptually, the regulatory system for chemical safety is a bad joke. That's because the scientific method (i.e. experiments--the system that chemical Q-RA pretends to use) is designed (biased) to avoid 'false positive' errors. Every experiment requires hard proof that the hypothesized effect exists at a significant level and therefore that the null or default hypothesis is wrong. This is a highly conservative system, designed to eliminate 'false positive' errors through the production of objective (independent & reproduceable) data.

False negative mistakes, on the other hand, *are seldom investigated* (i.e, results which don't reach a significant level of change (usually 5%) from the null hypothesis are assumed to be correct and typically receive no further testing); ***even though*** *they are exactly what we look for when we ask if chemicals are safe!* In short, ***our chemical safety testing paradigm assumes that chemicals are safe, instead of assuming they're dangerous!!***

Often, chemicals don't even have to be disclosed on a label (e.g. cleaning products, as long as they don't make a pesticidal claim (e.g."disinfects")! Personal care products--whose toxins we intimately expose ourselves to--typically must list all intentional ingredients; from greatest to smallest amount, but each one's amount isn't given, in the USA or most countries. Finally, chemical exposure also comes from millions of other products which use toxic chemicals in their production, including food—now a highly chemicalized process: from the field; through processing; to transport, storage and display.

A full-field ('cradle-to-grave') chemical regulation law was enacted in the USA in 1976, the Toxic Substances Control Act (TSCA). Though giving the EPA some powerful authorities to regulate chemical risks, it has such giant loopholes and unused authorities that it has proved useless in controlling chemical risks. Aside from immediatelly grandfathering from safety tests the 62,000 chemicals in commerce at the time of enactment, it has *required the EPA to approve seven new chemicals a day* on average(!);[222] without giving it the resources to test even these new chemicals for safety—much less review the safety of existing chemicals.

That statutorily-mandated 90-day limit on EPA's review of the risks of all new chemicals meant that it instantly became impossible to adequately review their safety; rather EPA was immediately forced to rely on screening models of the potential risk of chemicals, especially of molecule structure/biologic activity relationships; without actual chemical testing or even much model validation. ***In fact, EPA believes that these models fail to identify the very 'false negative' errors that TSCA was enacted to control!***[223] EPA annually receives some 2,300 Pre-Manufacture Notices (PMN) of the intent to manufacture or import a chemical above a minimal amount. PMNs contain only the initial amounts of the chemical and superficial worker exposure potential. EPA is not notified when this changes (except on the rare occassions when EPA has asked for such updates). Therefore EPA's is an utterly superficial review relying largely on the simplistic assumption that molecule structure predicts toxicity—an approach still relied on after both the 1994 and 2005 GAO reviews found that to be an unreliable indicator. In any event, only 10-15% of the new chemicals so screened are flagged for any further investigation,[224] and almost invariably they are allowed on the market anyway. So, even acting as a gatekeeper of new chemical safety, EPA has insufficient data (more important, that data is typically contradicted...after the

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[222] Env. Working Group (EWG) July 2005 'Body Burden: the pollution in newborns' (Exec. Sum.; accessed 17 Jul. 2005 at: http://www.ewg.org/reports/bodyburden2/newsrelease.php).

[223] General Accounting Office June 2005 'Chemical Regulation: Options Exist to Improve EPA's Ability To Assess Health Risks & Manage Its Chemical Review Program', Wash. DC.; avail. At: http://gao.gov/cgi-bin/getrpt?gao-05-458. **Also**: General Accounting Office/RCED Sept. 1994 'TSCA: Legislative Changes Could Make the Act More Effective' GAO/RCED-94-103, Wash. D.C

[224] NTP Oct. 2004.

EPA allows the new chemical to be sold![225]).

On the infrequent occasions when EPA decides it wants more data before allowing a chemical on the market, it has for three decades to date refused to issue rules requiring such data. Instead, it enters into "enforceable consent" agreements with the producer/importer…despite at least one district court ruling that TSCA mandates that rules (which are much more enforceable) be issued to aquire such data (in response, EPA switched from voluntary agreements to the ammbigous "enforceable consent" agreements).[226] **In sum** for new chemical safety testing, EPA has only banned or restricted ***one*** new chemical family under TSCA!

For existing chemicals, EPA can only review their safety after a series of determinations of the necessity to do so, which an Appeals Court defined very stringently in creating that current standard. EPA initially (in 1979) reviewed (in some way) the risks of only 2% of the 62,000 grandfathered chemicals.[227] By FY 2000 the grandfathered list grew to 78,598 chemicals in commerce, and EPA had reviewed the safety of just 20.4%.[228]--mostly during *new* chemical review (i.e. based on manufacturer/importer's PMNs for new chemicals). **In sum** for existing chemicals, EPA has only banned or restricted ***five*** existing chemicals/chemical families(!), and taken minor action on a few thousand others.

Although EPA does have a chemical Inventory Update Rule (IUR) that requires production/import volume and location reporting every 4 years for the ~9,000 chemicals produced/imported in volumes greater than 10,000 lbs/yr (excluding inorganic chemicals), a Clinton Administration effort to expand the rule to report the uses and population exposures of the chemical[229] was abandoned. In fact, in 2003 the Bush-II Administration raised the IUR reporting threshold from 10,000 to 25,000 lbs, and created a threshold of 300,000 lbs/site for processing and exposure information.[230]

TSCA does require companies to notify EPA immediately after becoming aware of any data indicating risk of a chemical they produce/import, but the loophole here is that to be reported this data has to "reasonably" support the conclusion that it poses a risk to humans or the rest of the environment! Yet TSCA also mandates that information supplied by companies is confidential--an assumption that must be rebutted by EPA in order to release it. That is just the right atmosphere to diddle data in! EPA estimats that a large proportion of the data in PMNs is claimed as CBI

In summary for TSCA, the minimal toxicity tests that are required are performed, as always, by the party with massive economic interests in seeing it declared safe--the final Problem (#8, below) of chemical Q-RA.

Given that drugs and pesticides are the only chemicals in commerce whose sale is dependent on at least some safety testing , it is shocking to learn how superficial and useless such 'pre-market approval' tests remains, whether TSCA or otherwise-derived data: In 1984 the USA's National Academy of Science (NAS) determined that of a sample of 257 of the 2,800 to 3,000 high production volume (HPV; greater than 1 million lbs./yr) chemicals, only 20% had minimal health effects information (similar for 14,000 chemicals produced in lesser quantities). By type of chmical, the availability of even minimal toxicity data ranged from 44% for cosmetics to 25% for drugs).[231] In 1997 a different sample of 100 HPV chemicals said that 29% had sufficent basic safety data publicly available.[232] The chemical industry reponse said that 53% of those 100 HPV chemcials had sufficent basic safety data.

In 1998 the EPA both settled that argument; and acted to address (via voluntary measures only and for HPV chemicals only), the risks of already-marketed chemicals. First, analyzing *all* (not just a sample) of the 2,863 HPV chemicals, EPA determined that only 9% of HPV chemicals met the Organization for Economic Cooperation & Development (OECD, the rich-country's club)'s definition of a primitive (basic) safety testing data set, publicly available! In fact, 43% of all HPV chemicals had *none* of the six elements comprising that basic set of safety data, and only 7% of the chemicals had all the

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225 GAO/RCED 1994.

226 GAO-05-458.

227.GAO/RCED-94-103.

228 EPA/Office of Chief Financial Officer accessed 30 July 2003 'Goal: Preventing Pollution & Reducing Risk In...', http://epa.gov/ocfopage/budget/2002/2002final/04_11.pdf.

229 General Accounting Office/RCED July. 1995 'TSCA: EPA Should Focus Its Chemical Use Inventory on Suspected Harmful Substances' GAO/RCED-95-165, Wash. D.C. (originally withheld, later released by the GAO).

230 GAO-05-458.

231 Ntl.Academy of Scieces/ National Research Council 1984 'Toxicity Testing: strategies to determine needs & priorities, Wash. D.C., Ntl. Academy Press.

232 Environmental Defense 1997 'Toxic Ignorance: the continuing absence of basic health testing for top-selling chemicals' -- http://www.scorecard.org/chemical-profiles shows the testing status of thousands of top-volume chemicals.

basic safety data (or 8%, when counting some that were finished, but not yet publically available)![233] (OECD's estimate was that half of HPV chemicals met none of their six basic categories of safety data).[234]

However, EPA's analysis--the only one to analyze all HPV chemicals--is an admitted "best case". It assumes that the existance of a test methodology & result is a complete and honest one; rather than evaluating the quality of the data (see Problem #8). EPA also admits that it counted as complete data if there was only one test, when in fact the OECD requires at least one confirmation of a safety result.

Cancer has always been the main health endpoint examined by chemical Q-RA. 10% of chemicals in commerce show some evidence of carcinogenicty.[235] Despite those two facts, just 2% of the chemicals in commerce have had their carcinogenicity adequately evaluated.[236] Even fewer chemicals have been tested for effects on other health endpoints. These cancer and non-cancer data gaps for each individual chemical have been documented repeatedly, even among chemicals with the highest use.[237] In fact, the number of chemicals tested even paritally (by the NTP) has never managed to close the gap created by the total number of chemcials in commerce, and is not projected to anytime soon (an all but infinite number of potential chemicals exists, and so far industry has been introducing new ones faster than they are even minimally tested).[238] It's been said that just 15 chemicals have been tested for neurodevelopmental toxicity![239]

PROBLEM 7: ALMOST NO EXPOSURE AND DISEASE MONITORING Another major flaw of using Q-RA as the basis of chemical risk management is that society has almost no reliable monitoring/feedback systems, such as personal exposure measurements and the disease & ecologic effect registries necessary--in a world with many complicating and confounding factors--to assign or to indicate causation. For chemical risks, we are flying, almost literally, blind (a circumstance that business executives, doing their job, would never accept--they know the critical importance of feedback to managing). *This problem is elaborated on in the next section*.

PROBLEM 8: DIDDLING DATA (THE ECONOMIC INCENTIVE TO PROCLAIM "It's Safe") ***Even if chronic toxicity testing, the heart of chemical Q-RA, somehow overcame its above seven fatal flaws, the problem of economic conflict of interests (CoI) remains*: the huge profit to the sellers of these chemicals drives them to proclaim them safe.** The safety testing of chemicals is entrusted largely such corporations, whose legal mandate is to maximize short term profit. Again: that venal failure is independent of the incompetence of chemical Q-RA to assure safety. Corporations and their officers and staff--with a fiduciary trust legal duty to maximize the returns to shareholders at the expense of anything else--in fact have a second fiscal incentive to manipulate science until it allows them to act freely: freedom from the constraints of tort actions against them for harming others or property.

Altogether, "Ignorance is bliss" and information asymmetry both give power (from regulators and competitors).[240] There seems to be no other area of society where information is so controlled than in chemcial and environmental risks.

Environmental laws obviously assume that enough is known about chemical risks to protect health and the environment. However--even if all the problems of chemical Q-RA vanished--the problem of hidden or manipulated data would remain.

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[233] U.S. EPA/OPPT Apr. 1998 'Chemical Hazard Data Availability Study' http://epa.gov/opptintr/chemtest/hazchem.pdf, accessed 12 July 05.

[234] OECD April 1990 Press Release, Paris France.

[235] D. Carpenter et al. 2002 'Understanding the Human Health Effects of Chemcial Mixtures' Env. Health Persp:110(Suppl 1):25-42.

[236] V. Fung et al. 1995 'The Carcinogenic Assay in Perspective' Env. Health Perspectives:103:680-3.

[237] R. Allanau et al. 1999 'Public Availability of Data on EU High Production Volume Chemicals' Ispra, Italy: Joint Research Center, European Commisiion. **Also** U.S. EPA/OPPTS 1998 'Chemical Hazard Data Availability'.

[238] R. Thomas et al. (Dec. 2002).

[239] Amer. Association on Mental Retardation (AAMR) 2004 'Pollution, Toxic Chemicals & Mental Retardation--proceedings of a national Wingspread summit' Wash, DC; but they do not cite their source.

[240] Wendy Wagner Apr. 2004 'Commons Ignorance: the faiure of env. law to produce needed information on health & the environment' Duke Law J.:53:1619-1746 (updated version: http://www.law/duke/edu/journals/dlj/articles/dlj52p1619.htm (accessed 2/'05).

But these laws also have problems of their own in producing even some of the necessary data from companies (who are a natural source of much toxicity and exposure information).[241] These laws do not mandate that companies pay for the independent production of unbiased risk data. Instead, their requirement that companies turn over to government any knowledge of risk creates strong incentive for the parties to disown such knowledge, because doing so leads to regulation of their activities. Court interpretations have firmly established that the goverment has the burden to absolutely prove that a risk proven before chemical regulation occurs. [To try to bypass all these problems, environmental laws took to mandating the best achievable emisision levels--a method with its own problems, as the companies also control the cost and performance data of these technological control methods.]

There is oft-replicated empiric data that correlates the results and conclusions of industry scientists (when its even of publishable quality!) with the opinions and profit-motive of their sponsors, a phenomenon named the 'funding bias'. There're too many papers to cite (e.g. involving smoking, asbestos, many pharmaceuticals, and various widely used single chemicals); but at least two have found that effect widespread in the area of industrial chemicals.[242]

Critically, it has been decisively shown that government oversight of the quality and independence of privately-funded research is much poorer than its oversight of publically-funded research--even as goverments use for-profit research in regualtions meant to protect environment, health and safety! (except for the limited prohibitions on witholding/terminating studies showing adverse effects).[243]

This ever-present conflict occasionally is exposed to sunlight in crisis such as test lab scandals. In 1991, following the test-fraud scandals described below in the critique on pesticide registration (because they more-or-less largely concerned pesticide tests), EPA's Inspector General severely criticized EPA's *new* oversight of the labs doing chemical regulatory testing.[244] Even worse, an outside investigation reported that EPA has *still* never done as much as a walk-through inspection of 76% of the labs doing studies on pesticides for manufacturers; and it reported that only 3.5% of the pesticide studies submitted to EPA are audited.[245] Even after that public finding, the Associated Press recently reported widespread environemental lab fraud--by corporations, victimizing laws and government agencies.[246] In fact, lab test scandals are exposed periodically, if infrequently; such a recent one involving over 2,000 customers in Canada and N. America.[247] On 24 Feb. 2005, the former owner of the laboratory Enviro-Comp (Baton Rouge) was sentenced to 46 months in federal prison for lab fraud (submiting false results to a client for analysis never performed)…actually committed while awaiting sentencing for a convictions on a previous lab fraud!! Such behavior is the inevitable result of our chemical risk assessment system--a "do-it-yourself" system that fails to allow for the fact that the party that performs the safety tests has a massive economic conflict of interests.

This Fagin & Lavelle investigation reminded us of a Center for Public Integrity study finding that 18 of 40 top level staff in EPA's major Office of Pollution Prevention & Toxic Substances (OPPTS, which includes pesticides and TSCA) over15 years went to work for the chemical industry and its associations, while only 2 went to work for public interest groups. It also reports that when EPA offered an amnesty to all chemical manufacturing or importing companies (including pesticide manufacturers/importers) in 1991-92, 11,000 toxicity studies were submitted by these companies that show risks and health effects of their chemicals (Under TSCA, FIFRA (the federal pesticide law) and other statutes, companies and people are obligated to submit knowledge of health and ecological effects to the EPA),[248] four times the number of studies submitted in the preceeding 15-year life of the law![249] The book also investigated the peer-review (refereed) published literature of four common toxic chemicals, including two widely used agricultural herbicides (alachlor and atrazine). 60% of the papers written by non-industry authors reported toxic effects. But 86% of the papers published that involved industry authors reported the chemical was safe--and that ignores the claims of safety that companies make in

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[241] Wagner 2004. See Secs. II-B; III-A.

[242] Bekelman JE, Yan Li and Gross CP 22 January 2003 'Scope and impact of financial conflicts of interest in biomedical research' JAMA 289(4):454-465; **and** Swaen GM & Meijers JM Sept1988 'Influence of design characteristics on the outcome of retrospective cohort studies' Br J Ind Med 45(9):624-9.

[243] D Michaels & Wendy Wagner 19 Dec. 2003 'Disclosure in Regulatory Science' Science:302:2073.

[244] Thomas Dawson 24 Feb. 1982 'Risk Consideration in Pesticide Public Policy Decision making', Wisconsin Public Intervenor office, Madison WI.

[245] Fagin & Lavelle 1997 'Toxic Deception: how the chemical industry manipulates science, bends the law and endangers your health', Common Courage Press.

[246] Larry Margasak 24Jan.2003 'Feds Say Private Labs Fake Env. Tests Jeapordize Enforcement' (c) Associated Press.

[247] Joan Walters February 24 2004 'An elaborate deception; Results routinely altered; sometimes a 'test' was done just by smelling a sample', Hamilton [ON] Spectator.

[248] Fagin & Lavelle.

[249] __?__ 4 Nov. 1996 'Agency Watch' Nat. Law J., p. A10.

studies that they're unwilling to submit to independent peers for review (it also ignores those science journals whose peer-review process is designed to get industry authors published[250]). After the book's 1997 publication, the EPA's Inspector General confirmed in 1997 the allegation that involved the EPA's role in toxic deception, according to one of its authors.[251] And in a very rare if not unique action, EPA fined Dow-Elanco $730,000 in 1995 for failing to report several hundred incidents of post-registration adverse effects from exposure to organophosphate insecticides and other pesticides.[252] Most recently, thousands more documentations of industrial fraud and deception concerning toxic chemicals have been revealed (mostly gathered through un-sealed trial discovery and depositions), many involving pesticides.[253]

Carcinogenicty has always been most heavily tested chronic toxicity health endpoint, and for decades two thorough testing programs have been regarded as the gold-standard for determing a chemical's carinogenicity--the USA's inter-agency Ntl. Toxicology Program and WHO's IARC (Int'l Agency for Registration of Carcinogens). However since the mid 1990's it appears that IARC has been downgrading the carcinogenicity of various widely-used chemicals, largely on the basis of unproven differences in mechanism of action between humans and test animals. It has become apparant that IARC's claims of limited industry representation on the IARC workgroups (which make the carcinogenicity determination) are lies. E.g., IARC claimed just 12 of 410 participants ever recieved support from the industries involved, whereas a U.S. NIEHS researcher claims that 30% of those workgroup participants, and 70% of the heavily-involved "observers", were industry-supported.[254] IARC wont release any workgroup member's declaration of conflict of interest, to help explain the discrepancy. Science literally withers and will die when it isn't transparant--in its results ("publish or perish") or in critical ancilliary information as this.

An assessment of corporate influence of health non-profits by Center for Science in the Public Interest found 140 non-profits and 30 university institutions currently accepting a high degree of largesse from corporations with interests that are largely opposite theirs![255] These corporations obviously want the public interest sheen of university's and health non-profit's to rub off on them, to disguise their harm-for-profit social contract. The growth of such arrangements in recent years is evident;[256] in fact, 15% of all random sampled papers of four major medicine and toxicology journals that *claimed* no CoI in their disclosure statement, *had* a financial CoI! 8% of those papers had CoI that very directly involved the subject of the papers--how those journals define CoI.[257]

In fact, such data diddling is a sign of the arrogation (take-over) of our society by private-interest corporations, who long ago realized that control of science and its academic insititions were critical to achieving such power. It is no coincidence that about once a century, dominant capitalism shifts its means of production--from the industrial age (steel), to the petroleum age (petrochemicals) and now the "life sciences" age (biotech and harmaceuticals--oops: pharmaceuticals).[258] Corporations have increasingly gained the power to harass objective scientists (e.g. harrassing subpoenas and scientific misconduct charges).[259] They have gained so much control that they force their defininition of"good science" (data that suits their interests) onto the insititutions of actual science, e.g. the recent federal Data Quality and Data Access Acts, which were wrtitten by industry lobbyists and passed with zero debate.[260]

Industry's self-interested attempts to diddle science are ceaseless. A new needs assessment on improving EPA's critical (very heavily-relied on) IRIS datatbase of the toxicity of chemicals reveals the irony that the American Chemistry Council (ACC, supported by the pesticide industry (the Amer. Crop Protection Assoc.)) wants to "share EPA's IRIS cost burden" [a

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[250] E.g. the journal Regulatory Pharmacology and Toxicology, according to seven respected independent scientists who signed a letter to its publisher (Academic Press) protesting the journal's peer-review process (see 19 Nov. 2002 press release from CSPI.

[251] Multinational Monitor Mar. '99, interview of Dan Fagin.

[252] EPA May 1 1995 'Press Release: EPA Fines Dow-Elanco for Failure to Report...' Wash. DC., reported in Cox 2002.

[253] PBS TV 2001 'Trade Secrets', an investigative documentary by journalist Bill Moyers, March.

[254] See the extensive correspondance exchanges, Vol. 9, #1 of Int'l J. of Occupational & Env. Health (more in v.8/#2).

[255] Center for Science in the Public Interest 2003 'Lifting the Veil of Secrecy' Wash. D.C., $15 at: http://cspinet.org.

[256] D. Blumenthal et al 1996 'Relationships Between Academic Institutions & Industry in the Life Sciences-an industry survey' NEJM:334:368-373; showing that such arrangements increased significantly from 1984 to 1994, at least in the university partnership subsector.

[257] M. Goozner 12 July 2004 'Unrevealed: Non-Disclosure of Conflicts of Interest in Four Leading Medical & Scientific Journals' Center for Science in the Public Interest, Wash. D.C.. http://cspinet.org/new/pdf/unrevealed_final.pdf .

[258] For a discussion, including citations, see the conclusion (titled 'The Implications') of the next major section (on the causes of chronic human disease).

[259] Wagner 2004. See Sec. III-C.

[260] Wagner 2004. See Sec. III-C.

direct paraphrase] by preparing the risk summaries entered into this all-important database! Yet when EPA asked the public what new chemicals should be in IRIS (or which need updating), industry fails to nominate any...except when they are disputing a chemical's toxicity with EPA (there's one partial exception). By comparison, other government agencies nominated well over a hundred chemicals in this latest IRIS needs assessment, even though only one public interest group responded to EPA's request for comments (compared to ten trade associations or chemical companies).[261] The ACC has also taken advantage of resource-limited government guardians of public health by siging cooperative agreements to help them do toxicology research, despite the obvious interest the private companies (the ACC)have in diddling the data that they thus gain significant control over. Both the National Instititue for Environmnetal Health Sciences (NIEHS, part of the NIH) and the EPA are in such agreements, with incomplete controls to shield them against such conflicts of interest.[262] This means, among other things, that in most peer-reviewed journals it will be impossible to distinguish these studies as independent from industry-funded; as often the only indicator of that is the 'source of support or funding' statement that is added to most published scientific papers.

Finally, **secrecy** is a reliable indicator of data gaps and data diddling. EPCRA is the only of the federal environmental statutes that requires that confidential business information (CBI) claims be substantiated (only because it is a "Right-to-Know" law!) Obviously, there's a tight correlation between trade secret and safety information about chemicals. The 1994 GAO TSCA critique also found that EPA staff believe that CBI claims, which stop data submitted to EPA from being disseminated) are excessive. In fact, a 1992 study by an industry consultant found that over 90% of PMN submitals contained CBI claims.[263] Chemical names, even company addresses are consistently claimed to be CBI; and compliance and inspection information is allowed to be claimed CBI. EPA never challanges CBI claims unless asked to.[264]

In addition to the trade secret loophole; other company records/plant inspections and past tort actions are partially to largely private.[265] State laws granting immunity from environmental violations proliferated in the 1990s, and the immunity appplies to reporting violations. And in addition to the continually growing power of fascism (the merging of corporate and state power), the new era of terrorism hysteria has already produced many cowardly laws from Congress, probably none more harnmful to transperancy than the "Critical Infrastructure Information" (CII) rule promulgated under the Homeland Security Act of 2002. The CII rule allows private parties to designate *any* information they want as relevant ot 'critical infrastructure', after approval by the agency whose mission it is to protect against terrorism, this information becomes confidential--even to federal and state agencies seeking to take actions. For good measure, it crimminalized even inadvertant release of CII by federal employees. Such additional broadenings seem to indicate that the rule was an industry initiative to use terrorism fear as guise to hugely broaden their privacy rights in the public sphere.

In sum, these narrow and broad data frauds offer glimpses into the reality of chemical safety testing. They do serve as goads to society to improve the reliability of this safety system. But as it is designed to allow business to be conducted with a minimum of regulation, it has yet to be abandoned for a more protective or precautionary risk system.

See our **additional examples** of pesticide-related test fraud, below (e.g. Congress' and EPA's action after IBT & Craven Lab scandals described below, as highlighted by the GAO and the EPA Inspector General); note that the labs involved in that endemic fraud were responsible for testing more than just pesticides.
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[261] EPA/ORD/NCEA Sept. 2003 'Needs Assessment for U.S. EPA's Integrated Risk Information System' doc. # EPA/635/R-02/004, Washington DC. See its Appen. C for all claims in this paragraph.

[262] U.S. Government Accountability Office Feb. 2005 'NIH & EPA Need To Improve Conflict of Interest Reviews for Research Arrangements With Private Sector Entities', GAO-05-191.

[263] GAO/RCED 1994.

[264] Wagner 2004. See Sec. III-B.

[265] Wagner 2004. See Sec. III-B.

Q-RA TRICKS THAT TURN 'RISKY' INTO 'SAFE'

1. CREATE & EMPHASIZE A NON-EXISTENT "MARGIN-OF-SAFETY:

A classic Q-RA deception is to substitute a metric called the "MoS" (margin of safety) for the non-cancer RfD 'safe dose' method. The MoS takes the RfD's built-in safety factors and explicitly calls them "safety margins", which are then highly emphasized and headlined in the text and in tables. The problem, described above, is that the safety factors of the RfD method are 100% arbitrary--they are not applied with reliable knowledge individual and species differences in response; and they certainly cannot compensate for missing toxicology data, which usually gets a weak 3 or 4-times the NOEL safety factor. For such reasons, the RfD metric does not advertise or emphasize that its calculated safe doses are in fact safe.

As an example of misuse of the MoS metric, assume that a Q-RA's estimated exposure that it evaluates happens to equal the herbicide's RfD, set with the usual safety factor of 100 times less than the NOEL. The MoS metric says instead that the Q-RA's estimated exposure has a "margin of safety" (MoS) of 100 times the NOEL. This gives the inescapable impression that the Q-RA is saying with certainty that the exposure is very safe, whereas the RfD metric is designed to leave the impression that the same exposure level is right at the margin of the estimated safe/unsafe exposure level. Moreover, the exposure scenario evaluated may be of an individual or a species (or both) that is more sensitive than the 100-fold "safety margin" granted.

Thus, the MoS trick causes a Q-RA's non-cancer risk estimates to appear about (depending on a herbicide's RfD's safety factors) 100-fold too low--not by changing the RfD, but rather by re-packaging it. In sum, not showing the RfD, while highlighting the presentation of the MoS, clearly shows that the intent of MoS metric is to create a false sense of safety.

2. USE *MULTIPLE NOELs* TO SEED DOUBT ABOUT STRINGENT SAFETY LEVELS:

A more basic and scientific fraud is when Q-RA's pretend a chemical can have more than one NOEL; i.e. multiple non-cancer 'no-effect' exposure levels! (But because cancer may begin from a single mutation to DNA, it's risks are legitimately calculated separately). For example, a Q-RA may list a 'systemic' NOEL (i.e. all chronic effects observable at a gross (visual or microscopic) level of observation) "NOEL" and a reproductive effect "NOEL". The point of finding a chemical's actual NOEL is to protect from *any* non-cancer chronic effect of the chemical. Listing multiple NOELs is non-sense, but it has the advantage of implying that the lower NOEL is not the real NOEL. A naive risk manager such as an agency bureaucrat might well think: "Hmnn--maybe the safe level of exposure is the average of the two...". In fact, listing multiple NOELs is an invitation to risk managers and policy makers to dismiss the putatively real, more sensitive NOEL. For example, say that an exposure estimate results in exceedance of one putatively true "NOEL" but not the other. Will a risk manager or policy maker--looking only for conclusions, as she is unfamiliar with the Q-RA methodology--believe that the herbicide's risk is only half as bad because only one of two "NOELs" was exceeded? Or will they understand that exceedance of the lowest "NOEL" is the only valid way to assess risk?

Even if a Q-RA properly uses only one NOEL, it is ridiculously easy to find fatal inconsistencies between different Q-RA's NOELs for a given chemical. A NOEL must obviously be the lowest in *all* the valid literature to be the real NOEL. There are so many parties performing chronic effect tests, with so many protocols and data gaps (the testing of so few doses and end-points), that it is almost always impossible to know whether a stated NOEL is in fact the actual NOEL. Below, in our critique of the individual Q-RAs relied on by this FEIS, are several examples of this problem.

In any case, as above, chronic toxicity tests for a NOEL only test unrealistically high doses; so even the lowest "NOEL" is almost never the true NOEL. Q-RA's failure to test at many and realistic, dose levels is one of its key frauds.

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Summary of critique of chemical Q-RA

The technical nature of chemical Q-RA discourages democratic decision-making (a key problem) even as it exudes the superficial glaze of being a scientific process—i.e. providing reliable answers. Chemical Q-RA pretends it can deliver a simple numerical risk estimate (usually not even a range--just a single number) to the unquantifiable question of chronic exposure risks.

Proponents of chemical Q-RA argue--often in a Q-RA itself--that chemical Q-RA is very conservative in protecting health. They say that many conservative assumptions are made as to toxicity and exposure. For example they emphasize that chronic toxicity tests give almost a lifetime's dose, while the real-life risk that the Q-RA is evaluating is a short, or at least infrequent, exposure.

This is true, but conceptually incompetent, *because no number of conservative assumptions can give us an answer to an un-studied question*. Defenders of chemical Q-RA almost invariably will try to hide the vast ocean of data gaps that their conservative assumptions are supposed to safely bridge. As to their widely-used preceeding example: why bother emphasizing that you're exposing a test animal for substantially longer than the than the human exposure that is being investigated, if you're not going to test the cumalitive exposures and actual exposure levels that actually occur (among the other deficiencies that we've described)? (Note also that these alleged "lifetime exposure" of chronic toxicity tests are exaggerated: the widely-used EPA/OECD chronic toxicity test guidelines allow less than half a lifetime of exposure[266]).

In fact, chemical Q-RA perform a risk estimate for separate exposure scenarios per chemical asessed. That escapes the responsibility for admiting*any* adverse effects, because each risk estimate pretends that the chemical is the only one contributing to a health risk. That is the consequence of the inadequacy of the cumulative exposure assumption.

So, to briefly summarize our critique of chemical Q-RA: chronic toxicity tests fail to test many widely-used chemicals (even one-by-one); they ignore the vast majority of potential health effects; ignore the most vulnerable and biologically active period of organisms--development; fail to monitor human exposure and disease; are done by the party with a huge economic incentive to declare safety; and--*perhaps most critically*--ignore the potential effects at the actual doses received from the multiple chemicals we are exposed to. Because it fails to even try to test the hypothesis it claims to test: what level of chemical exposure is safe, we have entirely proven our main claim: that *chemical Q-RA is a scientific fraud*.

More wholistic views of chemical Q-RA than our detailed critique also basically conclude that it is a fraud. The National Academy of Sciences (NAS) has said (short of calling it fraudulent):

> "Risk assessment techniques are highly variable and almost all rely on multiple assumptions of fact--some of which are entirely untestable."[267]

In fact, the NAS has identified at least 50 steps at which Q-RA that require 'professional judgement' in place of tested data(!)[268] assumptions that give the corporate users of Q-RA (or their captured government agency overseers) all the flexibility they need to achieve a result they can live with (after all, a corporation's chief purpose--and fiduciary mandate--is to maximize their profit).

There exists at least one quantitative, independent study showing that chemical Q-RA is such a fraud, and that it fails to protect us. Eleven European countries each told their scientists and engineers to--in conjunction with industry counterparts--evaluate the acute risks of a small ammonia plant. The 11 teams' independent estimates of this one risk varied 25,000-fold![269]

In addition, there are major differences in how 'conservative' the assumptions are. For example among the federal agencies of the USA, the Occupational Safety & Health Administration (OSHA) is decidely radical. Their dose/response assessment models use the median, not the upper-bound of the range of the estimated potency, and they assume that potency falls faster than the dose on their D/R curves ("sublinear" potency).[270] In fact leading carcinogenicty experts concluded in a broad review that different tests of individual chemicals varied in their estimate of the cancer potency (at

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266 EPA/OPPTS 1998.

267 A. Miller et al. 1991'Environmental Epidemiology, Vol. 1: Public Health and Hazardous Wastes' National Academy of Sciences, Wash. D.C. (p. 45).

268 Government Accounting Office Aug. 2001 'Chemical Risk Assessment, Selected Federal Agencies' Proceedures, Assumptions and Policies' Rpt. # GAO-01-810, Wash. D.C. (pg. 31).

269 Commission of the European Communities 1991 'Benchmark Excercise On Major Hazard Analysis'. Luxembourg City, Luxembourg (3 volumes).

270 GAO 2001, at pg. 38.

least at the low end of the dose range tested) ...by 3-4 orders of magnitude (1,000 to 10,000-fold)![271]

It is often said that a chemical Q-RA is the equivalent of society agreeing to shoot one innocent person (they'll die or survive) out of 10,000; or 1 in a million--whatever is society's "acceptable risk", for every chemical and every chronic disease caused. The obvious purpose of Q-RA is not to ensure adequate safety; it is to allow business to continue as usual.

As we emphasized, chemical Q-RA was largely a response to the organic chemistry revolution of the 19th and 20th centuries. The 'linear dose/response' paradigm of traditional toxicology (which coalesced as a discipline in the 1920's) contrasts with today's realistic biology, which recognizes that life is entirely composed of subtle and complex biochemical signaling. These competing paradigms of toxicity have been neatly if incompletely summarized as:

> "OLD: High level of contamination overwhelms detoxicification and other defense mechanisms.
> NEW: Low level contamination hijacks control of development.[272]

Enough experiments have shown classic toxicology to be all but useless to predict risk, yet policy and law haven't adapted to reality.

A MORE CAUTIONARY APPROACH

Altogether, a system more friendly to chemical producers and users than chemical Q-RA can hardly be conjectured, while still remaining nominally a democracy. In fact, the features of classic toxicology and of Q-RA risk management system were created with powerful private corporations (and their mandate towards self-interest) at the center of the decision-making, as we periodically show throughout.

In formal logic nothing in the universe can be completely known until everything in the universe is known: some unknown factor could potentially change anything that is currently accepted as irrefutable natural fact, such as the universality of gravity. But the universe, including our biosphere, is so complex that even practical "beyond-a-reasonable-doubt" causation is incredibly hard to establish (e.g., is smoking tobacco a known and potent carcinogen to the small cells of the lung epithelium?--that took decades to establish, in the face of determined opposition by the private tobacco industry).

Humanity's natural condition of indeterminacy in a complex universe makes it *imperative* that societies be allowed to take preventative action *in the midst of partial scientific uncertainty,* as the independent literature inexorably continues to gather the necessary evidence to seriously evaluate chemical risks. The cost/benefit ratio of allowing mistakes to continue (e.g., until we conclusively prove that smoking, or the widespread herbicide 2,4-D (another subject bogged-down for decades in controversy) cause cancer) is far too great.

Exercising precaution over chemicals is as simple as shifting the burden of proof for a consequential action (such as EIS' evaluate) from Q-RA's unconditional, unreasonable: 'must show it will cause harm to stop the action; to the precautionary principle's conditional and logical: 'must show it is safe enough for society to allow the action'. The ubiquity of scientific uncertainty (due, again, to the extraordinary complexity of the natural world) make the precautionary principle the only logical paradigm to manage risks with.

Basically intuitive, the precautionary principle has been admirably stated (some time after being featured in the Rio de Janeiro's 1992 Earth Summit declaration, signed by almost all nations) as:

> "...When an activity raises threats of harm to human health or the environment, precautionary measures should be taken *even if* some cause and effect relationships are not fully established scientifically...taking action in the face of uncertainty."[273]

Investigating alternatives, and increasing citizen's involvement in the decision-making, are the two final components necessary for the Precautionary Principle to work in a society.

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[271] Interdisciplinary Panel on Carcinogenicity Aug. 17 1984 'Criteria for evidence of chemical carcinogenicity' Science:225:4663:682-7.

[272] John Peterson Myers Nov. 2002 'From Silent Spring to Scientific Revolution' San Fransisco Medicine:? (http://www.sfms.org/sfm).

[273] C. Raffensperger & J. Tickner, eds. 1998 *Protecting Public Health & the Environment: implementing the precautionary principle*. Washington DC: Island Press.

Wheras chemical Q-RA in practice forces the public to prove absolute causation (in a world full of complex interactions!) that a chemical causes great harm before it can be taken out of commerce; the precuationary principle employs a moderate standard, similar to civil law's 'preponderance of proof' standard.

The Precautionary Principle presents complex questions about risk and benefit--as complex as any socio-technical ones--but consider that the questions that chemical Q-RA seeks to answer are unknowable.[274] No improvement in toxicology--say, fixing the important problem of testing toxicity at environmetally relevant doses--will *ever* be able to predict the consequences of our ongoing, exposure to an ever-changing complex mix of chemical (especially as millions of variables in the natural world are also in flux). *Far* more precaution is--irreducibly--the only reasonably safe chemical risk management option.

EIS' are a flexible tool that allow for precaution, should that prove necessary in the proposed action the EIS evaluates. **In sum**, a more cautionary and effective approach to chemical risks than Q-RA is fully warranted.

So ...DO TODAY's UNIVERSAL BUT LOW EXPOSURES CAUSE CHRONIC DISEASE?[275]

Having spent up to this point all our effort exhaustively proving that the all-important chronic toxicity test is a scientific fraud because it fails to test the risks of people's ubiquitous low-level exposures; and having exhaustively demonstrated the strong possibilty that everyday low exposures do often cause chronic toxicity to vertebrates and others; to conclusively prove the latter *we must **also** show* that organisms are chronically exposed to toxic chemicals.

EXPOSURE VARIANCE

More than any other arm of the petrochemical industry, the pesticide manufacturers love to claim that their second and subsequent generations of synthetic pesticides are not persistant and don't bioaccumulate, thus human exposure must be far less than for DDT and other now-banned or withdrawn pesticides. Certainly thousands of published studies document the body burden of persistent bioaccumulting toxic chemicals that every organism carries. The average non-occupationally exposed human in a developed country has dozens of pollutants in her body.[276] An untold number of others--but many, many--are not analyzed for. A recent study was the first to look at a wide range (n=89) of hormone-disrupting pesticides and other common consumer-use chemicals in a large number of homes (120), finding almost every one of these hormone disruptors in the dust and air of every home. Most often their concentration exceeded government safe levels (if there was such a standard; and even though these allegedly safe concentrations don't consider hormone disruption, among their many failures).[277] Urine levels of one resident in each of these homes will soon be reported.

However, given the infamous disasters caused by the first wave of highly persistent--even biomagnifying--synthetic chemicals (DDT, PCBs, etc.), many second generation organic chemicals (including many pesticides) were designed to be less persistent, by making them more reactive (they prefer we ignore that this makes chemicals more water soluble and thus mobile in the environment). Industry constantly claims that these newer chemicals rapidly degrade in the environment or are excreted rapidly and so can not pose risk to humans and other organisms in the middle or top of food chains, so their only risk is if exposure occurs immediately after their release into the environment (their most obvious risk is the biologic damage caused by their very reactivity).

Importantly however, many of these less persistant organics *nevertheless could* bioaccumulate up food chains--in fact all organics except those that are near-completely water soluble or those that are very volatile from soil to air could

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[274] Peter Montague 20 Feb. 2003 Rachel's Env. & Health News #763 (http://erf.org).

[275] If so, it is "like a nightmare carved in molasses." (unknown). **Or**: "If we are going to live so intimately with these chemicals, eating and drinking them into the very marrow of our bones, we had better know something about their nature and power." (Rachel Carson in Silent Spring, 1962).

[276] The Centers for Disese Control & Prevention (CDC) in recent years has begun systematically measuring several dozen toxins in a random representative sample of Americans (NHANES-III). The public interest group Environmental Working Group has recently measured an even greater number of toxins though in a smaller sample, see http://www.ewg.org/bodyburden .

[277] Ruthann Rudel et al. 15 Oct. 2003 'Phthalates, Alkylphenols, Pesticides, Polybriminated Diphenyl Ethers and Other Endocrine Disrupting Compunds in Indoor Air and Dust' Env. Science & Technology:37:20:4543-53.

bioaccumulate![278] Though a chemical's ability to persist is the main determinant of their presence at all levels of a food chain,.nevertheless that finding may contribute to the explanation of why relatively reactive, water soluble organics (e.g. the herbicides mecoprop, atrazine and 2,4-D) are so prevalent in humans and top predators.

Second and obviously, incessant high-volume production causes intimate exposure to even non-persistent and non-bioaccumulating synthetic chemicals (many examples, including phthalates or the herbicides 2,4-D or propoxur). Their endless production mimics persistence and bioaccumulation. In fact, many of the so-called "degradable" pesticides which that industry pushes are found in relatively pristine areas hundreds of kilometers downwind from heavy agriculture areas (more so when conditions are dry and reactive oxidants in the atmosphere are at low levels).[279]

Given those robust theoretical reasons, it's no surprise that the just cited CDC study of chemicals in a large and representative sample of the USA population found that 17 of the 23 pesticides detected (34 pesticides were looked for, along wiith other synthetic chemicals) were non-persistent ones or their metabolites--detected in urine, not in blood; and often at high levels.[280] In other words, every American's body is constantly processing these supposedly harmless non-persistent and non-bioaccumulating chemicals, often at levels above the inadequate safety standards that exist for those pesticides. Many additional studies document human body burdens of these less persistent chemicals, e.g. for pesticides alone.[281] 2,4-D is a frequent-industry cited example, so it is worth noting that it is found in the semen of exposed farmers and in the urine of exposed foresters.[282] In a random sample of 1,000 adults, 12% had 2,4-D in their urine. In fact, children's total dietary and post home-application intake of 2,4-D approached half of its RfD (non-cancer 'safe' dose),[283].not even accounting for the lack of tests on children or on many organs.

In toxicology even today, thorough metabolic studies are often not made until a problem is indicated--e.g. even for the ubiquitous toxin bisPhenol-A (bP-A), inter alia a common pesticide formulation ingredient--and now known to be an utterly potent toxin (see the example in 'Problem #1' above). Only now are the many metabolites of bP-A being discovered--far from having their influence on exposure and toxicity studied.[284] Certainly, the toxins found in any one compartment--urine, blood lipids, blood binding proteins, fat, the liver, etc.--underestimate the total exposure to all of an organisms functions.

Finally *(finally!)*, we are prepared to answer the fundamental question of chemical risks: are these real ubiquitous and low level chemical exposures causing disease in humans--much less in the rest of the biosphere?

Establishing causation—that A is an entire cause of B—even in by practical standard, in the phenomenal complexity of the world, is very difficult; given the innumerable variables in the physical universe. Nothing in the universe can be completly proved until everything in the universe is known. So while "the true method of knowledge is experiment",.[285] "causality is a concept not subject to empirical demonstration."![286]

Human society believes that it is wrong to try to control such variability in controlled experiments on humans (except for

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278 Gertie Czub & M. MacLachlan 15 Apr. '04 'Bioaccumulation Potential of Persistent Organic Chemicals in Humans' Env. Sci. & Technol.:38:8:2406-12.

279 DC Muir 2004 Oct 'Empirical and modeling evidence of regional atmospheric transport of current-use pesticides' Environ Toxicol Chem:23(10):2421-32.

280 Kristin Schaffer et al. May 2004 'Chemical Trespass: pesticides in our bodies and corporate accountability' Pesticide Action Network North America, San Fransisco CA (available: http://panna.org); which extracted the pesticide data from the CDC's less-accessible overall NHANES-III data.

281 R. Whyatt et al. (May 2002) Residential Pesticide Use During Pregnancy Among A Cohort of Urban Minority Women' Env. Health Perspectives:110:5:507-14; **and** J. Adgate et al. (June 2001) 'Measurement of Children's Exposure to Pesticides: analysis of urinary metabolite levels...' Env. Health Perspectives:109:6:583-90; **see also** EPA's Non-Occupational Pesticide Exposure Project (NOPES) project; **and** A. Bradman et al. Nov. 2003 'Measurements of Pesticides and Other Toxicants in Amniotic Fluid as a Potential Biomarker of Prenatal Exposure: a validation study' Env. Health Perspectives:111:14:1779-1782. .

282 ?, 1999 Reprod. Toxicol. 13:421-9; **and** V. Garry et al. May 2001'Biomarker Correlations of Urinary 2,4-D Levels in Foresters: genomic instability and endocrine disruption' Env. Health perspectives:109:5:495-500.

283 Nishioka et al. 2001 Env. Health Perspectives 109:1185-1191.

284 D. Zalko et al 2003 'Biotransformations of Bisphenol A in A Mammalian MOdel: answers and new questions raised by low-dose metabolic fate studies in pregnant CD1 mice' Env. Health Perspectives:111:3:309-319.

285 William Blake 1788 'All Religions are One'.

286 David Hume 1739 'Treatsie On Human Nature'.

the occasional natural experiment, and some ethically-impaired chemical producers--mostly pesticide makers[287]). Unfortunately therefore, in the study of human disease (epidemiology), the hardest cause of a disease to identify is the one that is universallty present(!),[288] as there is no direct way to distinguish it from other factors. While many experimentalists superfically believe that epidemiology fails to control enough variables to prove causation; many epidemiologists superficially claim that if their correlations of variables achieve relatively strict criteria, they have proved causation, or at least approached practical causation. The epidemiology criteria of Dr. Hill--the strength, consistency, specificity, direction, and plausibility of an association; plus a few more--are by far the most commonly used,[289] yet Hill himself emphasized that epidemiologic studies that fail to meet all or most of these critetia can still be critical in proving practical causation.[290] Epidemiologic studies adjust their correlation findings for all known variables, and have improved their exosure estimates. More and more are prospective, a requirement to show causation. In sum, practical causation requires independently-replicated positive findings in both epidemiology and in controlled animal experiments, plus a plausibly-detailed mechanism of action for the agent to cause the health endpoint.

Due to the USA's failure to establish disease or exposure population registries—which would greatly aid achieving those criteria--it is hard to gather enough data on even the major relevant variables to know what their influence is on the variable being investigated, and thus to approximate causation For example, it is rare in the highly-mobile population of the USA to track the source of drinking water over a lifetime. But the Scandinavian countries do track both human exposures and their diseases quite thoroughly. Thus, e.g., they have robust evidence that water chlorinated water disinfection byproducts (DBP) do cause cancers in people, and that is corroborated by other evidence.[291] Meanwhile, in experimental work, it is always possible to claim that species differences exist and therefore the results aren't relevant ot human risk (in general, however, the biochemical similarities between vertebrates are termendoous—see our discsussions of genetics and of cancer in 'Problem 1', above).

The chemical industry and other profiteers love to take advantage of this complicated and indeterminate universe of ours, and claim that chemicals and their other technologies have not been proven harmful, or that they cannot be proved harmful. Using science's standard of proof, they can demand 95% certainty that a checal is harmful or deadly before its use can be limited, even assuming they don't do the science themselves. Also, as stated, results aren't always clear, at least not immediately.

While spending tens of billions of dollars every year to raise all these doubts about chemical risks, the industry avoids adequate tests of potential risks; and where data is not obtainable prefers the burden of proof to be "prove that it is safe". While society could never prove absolute safety of inherrently safer alternative technologies, and while there will always be risks and benefits that society must balance, the critical principle is that ***'the absence of evidence is not evidence of absence*** [of an effect]'. Almost always you will find that industry's claims of safety are founded on 1) mammoth data gaps and 2) invalid (non-independent) experiments and studies. The classic example of these competing public vs. private interests is that toxicology took decades to overcome the real data gaps and the tidal wave of the tobacco industry's fraud & deceit, to finally practically prove with the weight of the evidence, that smoking causes small-cell lung cancer in many smokers.

To practically prove causation (i.e. beyond any reasonable doubt), robust, diverse and multiple lines of evidence are needed; colloquially called the "weight-of the-evidence" approach. Together epidemiology, controlled lab experiments (usually on fellow-mammals) and a plausible mechanism of action (the biologic steps by which the agent could lead to the disease) are the three categories in which evidence must be produced to practically prove causation. Evidence in each of the three areas must be replicated independently, and accepted for publication in a peer-reviewed journal (or at least other nominally non-biased medium, such as a government agency report). Logically, it is interesting that all causes of human disease are environmental, just as all are caused by our gentic make-up.[292] That is, we all have genes that

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[287] E. Shogren 27 Nov. '01 'Pesticides: EPA says it will accept industry data gathered by giving paid subjects chemical doses' L.A. Times. Dow, Bayer and others are leading this unethical initiative, driven by the desire to avoid the 10-fold safety factor for inter-species response variation applied to regulations.

[288] G. Rose 1985 'Sick Individuals and Sick Populations' Int'l. J. Epidemiol.:14:32-8.

[289] A.B. Hill 1965 'The Environment & Disease: association or causation?' Proc R Soc Med.:58:295-300.

[290] KJ Rothman & S Greenland 2005 'Causation & Causal Inference in Epidemiology' Amer J Publ Health:95(suppl 1):s144-s150.

[291] G. Boorman et al. 1999 'Drinking Water Disinfection By-Products: Review and Approach to Toxicity Evaluation' Env. Health Perspectives:107(Suppl. 1):207-219. The evidence that halogenated DBPs cause several cancers, birth defects and reproductive damage is overwhelming--hundreds of independent papers. Yet public health advocates have convinced the public that chlorination is their achievement of the century. In fact, halogens are very weak disinfectants compared to the far less toxic alternatives (its only partial advantage is that its disinfection lasts). Thus water disinfection is a perfect example of the failures of Q-RA and of the need for a precautionary approach (below).

[292] Rothman & Greenland 2005.

control our biology, but they co-exist with us in our environment (the universe).

Despite those dual problems in assigning causation (the complexity of nature and the USA's failure to track the incidence of chronic diseases), it has gradualy become impossible to deny that many of those incidences--which have little or no connection to other environmental factors such as smoking and diet--have increased significantly, even rapidly; in the industrial era; coincident with increased exposure to industrial chemicals. One initial line of evidence that the low-dose but ubiquitous chemical exposures (that the CDC has been documenting are in the bodies of all Americans) cause chronic disease is the recent strong correlation between pollution in different regions of Ontario and increased health care costs (and vice-versa): a difference between the cleanest and most polluted regions in health costs per person-year of $355; after controlling for variables known to affect health care expenditures, such as income and education. Measuring a similar variable--pollution control expenditure--the difference was $200/person/year of health care cost.[293]
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TREND IN CHRONIC DISEASE INCIDENCE: ***UP OR DOWN?***

That human life-spans are growing (thus more opportunity to acquire disease) would bias trends (i.e. over time) in disease incidence...were it not for the fact that the incidence rates are adjusted for just this factor! Next, many of these chronic diseases that are or seem to be increasing, it will be seen, are obvious diseases, so better technology that aids detection is not a factor (debunking another argument that incidence rates are falsely increased). A significant degree of better detection and reporting has occurred for many of the less or non-obvious diseases, falsely inflating their incidence rates; but not in the past 30-35 years--which is enough time to see what the true incidence trend is for many such non-obvious diseases.

Moreover, for the non-obvious chronic diseases, causes of death (often more than one cause contributes and may be listed) are often mis- and especially under-diagnosed. Autopsy is the best way to determine causes of death (with a 1-5% failure rate), **but there's been a long decline in autopsy rates**, fueled by fear of malpractice litigation, cultural customs, and other socio-economic factors. A recent systematic analysis of the literature on autopsies and reported causes of death has discovered (controling for publication bias) that while the rate of error (in cause-of-death asssignation) has steadily declined, so has the rate of autopsies; leading to only a minor decrease in errors in cause-of-death since 1960 in the USA.[294] The direct implications to health are serious [e.g. the authors estimate a major diagnosis is missed in 8.4% of USA deaths (71,400 people a year); of which 34,850 are likely to have survived (at least to discharge from hospital). Although a speculative estimate, the latter number is validated by the separate estimate by the Institutes of Medicine, that medical errors overall (including many drug dosing errors) kill 44,000-98,000 Americans a year]. In any case, the main cause-of-death error is to miss a disease, not to mistake it for another. Therefore the decline in autopsy-confirmed cause of death has another, indirect, effect--it causes a significant under-estimate of the incidence rates of chronic diseases (in addition to not tracking the incidence rates in the first place!).
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INCIDENCE TRENDS--CANCER (and Birth Defects)

Cancers and most types of birth defects are the two endpoints that *do* have reliable incidence tracking in the USA, and they are mostly obvious conditions. These USA incidence registries show that the incidence of most types of these two disease categories has been rapidly increasing in this chemical age.[295], [296] In fact, in our high-technology medical approach to delay death from chronic disease, cancer deaths have for decades remained stubbornly unchanged, only dipping 2.7% from 1970 to 2002.[297] Thus cancer just overtook heart disease as the leading cause of death in the US for those younger than 85 (for over a century heart disease killed far more than did cancer, but all that time its death rate has been in steep decline). For the oldest Americans, heart disease still kills far more than cancer, yet their cancer mortality

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[293] Jerrett M. et al. 2003 May 'Environmental influences on healthcare expenditures: an exploratory analysis from Ontario, Canada.' J Epidemiol Community Health.;57(5):334-8.

[294] Kaveh Shojania et al 4 June 2003 'Changes in Rates of Autopsy-Detected Diagnostic Errors Over Time' JAMA:289:21-2849-56.

[295] NCI 1994.

[296] Larry Edmunds et al. Dec. 1990 'Temporal Trends in the Prevalence of Congenital Malformations at Birth Based on the Birth Defects Monitoring Program, U.S. 1979-1987' Morbidity & Mortality Weekly Report 39:SS-4, pg. 22, CDC. (This BDMP database samples ~15% of the live births in the U.S.).

[297] A Jemal et al. 14 Sept. '05 'Trends in the Leading Causes of Death in the United States' JAMA:294:1255-9.

is actually rising a bit·[298]. Obviously, only prevention--not early detectin or cures--can reduce the tragedy of cancer.

A meta-analysis of cancer mortality world-wide shows congruent increases among industrialized countries (at least those with registries reliable enough to adequately measure the trend), even when excluding lung and stomach cancers (to exclude much of the large influence of tobacco and diet).[299] The International Agency for Research on Cancer (IARC, part of the World Health Organization and one of two 'gold-standard' bodies evaluating the carcinogenicity of compounds) says that even recently (1990 to 2000), cancer incidence increased a full 19% worldwide (and 44% of the 900 agents that IARC has evaluated to date have been classified as possible, probable or actual human carcinogens).[300] Additionally, most cancer types arise more frequently in the industrialized world than in the less developed world--a finding replicated in 2000.[301]

Many cancer types have increased drastically in incidence; relatively few have decreased. Incidence of childhood cancers in the USA, for example, has soared 31% since standardization of the data: from about 130 to 170 per 100,000;[302] including a 56.5% increase in brain/CNS childhood cancers and a 69% increase for immune cancers.[303] Among children through age 19, the overall increase from 1973 to 2000 was 22%.[304] Testicular cancer soared 66% from 1975 to 2002. Breast cancer increased 25.3%, 1975-'96. Prostate cancer incidence nearly tripled 1973-'92, and is still 2.5X its 1972 rate. .[305] Breast cancer including DCIS now strikes at least one in six(!) women of the USA, up from 1 in 14 in the 1960's.[306]

In fact for all cancers, lifetime cancer risk in the USA is now almost one-in-two for men (45.59%) and has passed one-in-three for women (38.18%).[307] In the decade since reaching (after a steady climb) this high level, U.S. cancer rates have remained there: recently the U.S. National Cancer Institute (NCI) reported that an apparent recent small decrease in various prevalent cancers since the peak incidence was reached **in fact had not occurred**. Accounting for first time for corrected errors and slowness in reporting cases to our national cancer registry (SEER, the NCI's Surveillance, Epidemiology and End Results program), the incidence rates for the five most prevalent cancers (e.g.) jumped 3% to 14%. These incidence rate increases are 0.6% to 4.1% *per year* for these five cancers,1981 to 1998 (all statistically significant increases); while before the adjustments these cancers showed no significant increasing annual trend over the same period.[308] Even worse, no adjustment can capture cancers that are never reported, e.g. as autopsy rates continue to decline (see above). SEER does constantly seeks to improve case reporting, and its accuracy was recently validated in an accidental analysis of a random sample of cancerous tissues available for confirmation by independent pathologists.[309] In short, SEER--a huge, normalized and standardized national sample--demonstrates that many cancer types have now exploded in incidence. E.g. most childhood cancers are now epidemic when previously they were rare. (Note: SEER excludes a very common cancer, non-melanoma skin, as it is rarely fatal).

Regarding this fundamental fact--that half of Americans will get cancer: one of us recalls, growing up in the 1960's that the mass media would report with hysteria that one-in-four Americans would get cancer in their lives. This ubiquitous but annectdotal evidence is supported by analysis of SEER: from 1950 to 2001, overall cancer incidence in the USA increased 85%.[310]

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[298] A. Jemal et al. 2005 Jan-Feb 'Cancer Statistics, 2005' CA Cancer J Clin:55(1):10-30.

[299] Devra L. Davis et al 25 Aug. 1990 'International Trends in Cancer Mortality in France, ...and the USA' Lancet:336:478-91 (correspondance exchange 336:1262-5).

[300] Cogliano V. Sept. 2004 'The Science and Practice of Carcinogen Identification and Evaluation' Env. Health Perspectives:112:13:1269-74.

[301] International Agency for Research in Cancer (IARC) 2001 'GLOBOCAN 2000-Cancer Incidence, Mortality & Prevalence Worldwide, v. 1.0' IARC Press, Lyon France; available at http://www.dep.iarc.fr/globocan/globocan.html (accessed Jan. 2004).

[302] Tracey Woodruff et al. Apr. 2004 'Trends in Env. Related Childhood Diseases' Pediatrics:113:4(suppl):1133-40.

[303] Ntl. Cancer Institute (NCI) 2005 'SEER Stat Database: Nov. 2004 Sub (1973-2004)', available: http://www.seer.cancer.gov .

[304] NCI 2005.

[305] NCI 2005.

[306] L Ries et al., eds. 2004 'SEER Cancer Statistics Reviews, 1975-2001' Bethesda MD:Ntl. Cancer Institute; available: http://www.seer.cancer.gov/csr/1975_2001/ .

[307] Jemal et al. 2005 Jan-Feb.

[308] L. Clegg et al.16 Oct. 2002 'Impact of Reporting Delay & Reporting Error on Cancer Incidence Rates & Trends' J. Ntl Cancer Institute:94:20:1537-45.

[309] R Field et al. 21 July 2004 'Lung Cancer Histologic Type in the [SEER] Registry vs. Independent Review' JNCI:96:14:1105-7 (explained in editorial: E Henson & J Albores-Saavedra 'Checking up on the [SEER] Program').

[310] Ries et al. 2002.

Thus spurred by the possibility of hurdling the assumed barrier that insufficient data collection existed before 1973 (after all, statistical methods were employed by our best epidemiolgists long before then!), it occured to us to check what the NCI might have said about cancer incidence at its inception, 1937. Congress had directed their new NCI to disseminate research, for which the Journal of the NCI (JNCI) was created, first publishing in 1940. Sure enough, the fourth-ever issue contains an extensive paper and discussion attempting to estimate the incidence of cancer in the USA![311]

First, based on Public Health Service studies, overall cancer mortality appeared to have increased steadily over four decades (i.e. from 1900)--indicating that cancer rates were rising in this industrial urban environment (a period in which treatment success--a variable in time trends--presumably did not change much). Of course, this early increasing trend is somehat overstated by not adjusting for the population living to a longer average age, thus acquiring more cancers than they would have in earlier decades. Second, based on thorough surveys of physicians in 12 large metropolitan areas, the rates of cancer cases under treatment (a prevalence estimate) were estimated to average 363 per 100,000 of population, or 0.36%. If we assume a 70 year average lifespan and assume that treatment lasts a year or less, that should be a 25.4% lifetime risk of cancer in urban USA cities in 1940. That is, almost twice as many of us now acquire a cancer in our lives than we did in1940, after adjusting today's rate downwards to compensate for our longer lifespans. This is a strong proof that modern changes in our environment cause cancer. Note that more modern (SEER-based) incidence trend studies generally exclude respiratory cancers so that the smoking pandemic will not confound the trends.

Subsequent NCI reports generally supported an increasing cancer incidence in this country. Before SEER standardized cancer mortality and incindence reporting, the NCI oversaw three national cancer surveys (NCS). In a 1978 analysis emcompassing all three NCS and covering 1935 to 1974, did find generally decreasing incindence trends, after separating out lung cancers and non-white incidence rates (their steadily increasing incidences, yet still not beyond the rates of whites is certainly due in large part to improved diagnosis). Yet many types of cancer showed increasing trends over this period.[312] Another analysis, adjusting for the transition to SEER's standardized cancer registry in 1973 (e.g. for population aging), again found increasing incidences (1969 - 1976) for most cancer types;[313] as did another study analyzing 1947 to 1984 (for comparability over time, whites only and all from the same cities).[314]

In sum, the various NCI studies generally find increasing rates of many cancer types, but none analyzed overall lifetime risk of cancer until the NCI's more famous 1994 report (above); while the NCI's 1940 report does--and it indeed indicates that the overall cancer incidence rate (even excluding the pandemic of respiratory cancer) climbed dramatically during the Chemical--the 20th--Century.

In fact, the NCI—our best cancer researchers--knew full well from as early as the 1940's(!!), that our chemical age was producing an environment inimical to life. Senior NCI scientist Wilhelm Hooper warned in 1948 that our new industrial environment was causing a silent (as we aren't investigating it) wave of cancer, and implied that therefore we should take precautionary measures against toxic exposures.[315] By 1964, he was warning Americans that that represented an "[uncontrollable] epidemic in slow motion".[316]

When the European Union first standardized reporting to capture all childhood and adolescent cancer cases, they found also found that incidence increased 1% a year from 1970 to 2000 (and 1.5% a year for cancers in adolescence), and found that the increase was accelerating.[317] Neither improvements in diagnosis nor case registration were believed to be the cause, after a correspondence exchange. That they detected a convergance of childhood acute lymphoblastic leukemia incidence, towards the peak at age 2-3 that is typical only in in developed countries;[318] supports environmental causes for the increase.
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311 Selwyn D. Collins et al. Aug. 1940 'Trend and Geographic Variation in Cancer Mortality and Prevalence, ...' JNCI:1:4:425-50.

312 Susan Devesa & Debra Silverman Mar. 1978 'Cancer Incidence & Mortality in the U.S.: 1935-1978' JNCI:60:3:545-71.

313 E. Pollack, J. Horn May 1980 'Trends in Cancer Incidence & Mortality in the U.S., 1969-76' JNCI:61:5:1091-103.

314 Susan Devesa et al. Oct. 1987 'Cancer Incidence & Mortality Trends Among Whites in the U.S.: 1947-1984' JNCI:79:4:701-70.

315 Hueper WC. *Environmental Cancer*. Washington, DC: National Cancer Institute, Cancer Control Branch; 1948, 19pp.

316 Hueper WC, Conway WD. *Chemical Carcinogenesis and Cancers*. Springfield: Charles C. Thomas; 1964. More extensive quotes from the '48 & '64 reports are on pp. 27-8 **of**: R. Clapp et al. Sept. 2005 'Environmental & Occupational Causes of Cancer', written for the Cancer Working Group of the Collaborative on Health & the Environment.

317 Eva Steliarova-Foucher et al. 11 Dec. 2004 'Geographical Patterns & Time Trends of Cancer Incidence & Survival Among Children & Adolescents in Europe Since the 1970s (the ACCIS Project): an epidemiologic project' Lancet:364:2097-105.

318 Catherine Cole 11 Dec. 2004 'Registering Childhood Cancers' Lancet:364:207?-? (2 pg Editorial accompanying the above paper).

EXTRINSIC OR INTRINSIC CAUSE OF CANCER?

Next, there is little reason to believe that inherited genes cause much chronic disease, because it would be obvious that it runs in families--as it does in those well-known diseases and syndromes that are known to be inherited.. Roughly, exogenous and endogenous sources of disease may be labelled 'genetic' and 'environmental' causes respectively, though nature is so interwoven[319] that every single case of disease involves both (see our previous discussion). 'Environment' thus includes factors such as behavior, including diet.

Diet is obviously an important factor in disease. There seems to be consensus that in the ~10,000 years since human agriculture and animal husbandry began, the composition of the human diet has changed faster than evolution has adjusted to it, given the lifestyles of the preceeding 6-8 million years of hominin (hominid) existance--an apparent cause of much human disease today. It's also obvious that dietary changes accelerated with the industrial revolution, given the large increase in available food energy (enabled by reckless energy consumption), and the huge changes in the transport and processing of food. In short, changes in intake of fiber, carbohydrates (starches & sugars), fats, proteins, micronutrients (vitamins & minerals), sodium:potassium ratio, acid-base balance and cooking temperatures all cause human disease,[320] especially in the Western diet.

Indirect but intriguing evidence supporting environmental over genetic causes of change were discussed in the 'epigenetics' section of our chemical Q-RA "Problem #1", above: twins born with identical genomes who lived apart and had different lifestyles had more epigenetic changes to their genome as they went through life than did twins with similar lives.[321]. Another study found no more than 50% (schitzpophrenia) down to 20% and less (breast cancer, stroke & arthiritis) of identical twins developed those chronic diseases concordantly.[322] So, "identical" twins are not so identical after all' at least as they navigate through the environment of their life. Whether these differences in identical twins are due to epigenetic, genetic, or other parts of cells, their disease difference is almost certainly is almost certainly due to their environment, rather than inherited.

Also, many epidemiology studies have found that infection risk factors are also associated with risk of various human cancers[323] (given the strong links between immune function and cancer); so such studies, looking at exposure in time and space, may be a surrogate for associations of cancer with environmental factors in general.

Skeptics of chemical carcinogenicty--including almost every financially self-interested party (chemical manufacturers and users)--invariably rely on a 25 year-old estimate of the preventable (i.e. environmental) causes of cancer in the USA (and their trends); the famous & infamous "Doll & Peto" study. It estimates that occupational and environmental exposures to industrial chemicals cause about 7% of USA cancer.[324] Doll & Peto also estimate that some 2% of cancers are caused by workplace exposures, but a review of more recent evidence says that 10% is a more reasonable estimate.[325] That is one hint that Doll & Peto's estimate of all avoidable causes of cancer--though originaly an improvement in estimates--is not just outdated, it is severely flawed.

First, it analyzes the data of only the major cancers, so not only does fail to count a significant number of environmental cancers, it also ignores some cancers whose etiology strongly suggests the involvement of chemicals (e.g. cancers of the immune and CNS). It also did not count cancers in small workplaces, workers older than 65, or indirect exposures to workplace chemicals.[326] Importantly, Doll & Peto say that cancer *mortality* data is more complete and reliable than cancer *incidence* data, thus their use of cancer mortality data is an adequate surrogate for measuring cancer incidence. Perhaps this was true at the time, it definitely is not today, the result of both major improvements in tracking cancer incidence (e.g. the SEER database samples a third of the entire population of the USA) and of the degradation in tracking

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[319] Judith Stern, UC/Davis geneticist, observes: "Genetics loads the gun; environment pulls the trigger."

[320] Loren Cordain et al. 2005 'Origins & Evolution of the Western Diet: health implications for the 21st century' Amer. J. Clin. Nutrition:81:341-54.

[321] MF Fraga et al. 2005.

[322] AHC Wong1, II Gottesman & A Petronis Apr. 2005 'Phenotypic differences in genetically identical organisms: the epigenetic perspective' Human Molecular Genetics:14(suppl_1):R11-R18.

[323] **McNally RJ** 2005 Dec 'Is there a common aetiology for certain childhood malignancies? Results of cross-space-time clustering analyses' Eur J Cancer.;41(18):2911-6. **and Houben MP** 2005 Dec 'Space-time clustering patterns of gliomas in the Netherlands suggest an infectious aetiology Eur J Cancer:41(18):2917-23 **(twosequential papers by same team)**.

[324] R. Doll & R. Peto June 1981 'The Causes of Cancer: quantitative estimates of avoidable risks of cancer in the United States today' JNCI:66:6:1192-1288. **Also published as** an Oxford U. Press paperback **and as** the background (i.e. detailed) report to Congress's (Office of Technology Assessment) 'Technologies for Determining Cancer Risks Form the Environment' assessment, Wash. D.C. U.S. Govt Printing Office, 1981

[325] Claoo et al. 2005.

[326] Clapp et al. 2005.

causes of death (above). Therefore since Doll & Peto (1981), SEER has shown that the incidence rate of many cancers in the USA have exploded or grown steadily (as shown above).

Analyzing trends over time of various cancers Doll & Peto have to make a further claim: that improvements in survival rates over time are illusory. Therefore using mortality as a surrogate for incidence is *not* biased against showing increased incidence over time. They mention in passing (in their conclusion) two grounds for this claim. Certainly it is possible that there is more complete reporting of cases of cancer survival. But we do not see the logic of their second argument, apparantly that there is increased survival reporting for obvious or non-obvious cancers. It seems to us that, all things considered, it's likely that survival rates have increased over time, meaning that using mortality to estimate incidence would be biased against an increases in cancer incidence over time. If they have a superior argument, they do not make it.

Finally, although Doll & Peto demonstrate the unrealistically high exposure estimates used in a competing estimate of the environmental causes of cancer,[327] they cannot gainsay the fact that that study's number of cancers estimate was due to just six chemicals! [Note too how Doll & Peto argue against the possibility of cancer from low doses, which in more recent years has gained much credence (see our critique of chemical Q-RA)].

Studies have proven that inherited genes cause only a small fraction of cancers--e.g. the well-known tendency of immigrant populations to acquire the cancer types common in their host country;[328] or, in large studies of twins, often only one gets cancer.[329] Recently the U.S. Ntl. Cancer Institute (NCI) estimated that the environment (including diet and smoking) causes *at least* 80% of cancers in the USA,[330] similar to other estimates and refuting the infamous 1981 Doll & Peto estimate (below). In fact, adopted children whose parents died of a cancer before age 50 had a greater than five times chance of dying early from the same cancer than the overall population did, clearly showing that environment is a far more powerful causative effect of cancer than inherited DNA mutations (a similar but weaker pattern was observed for cardiovascular disease, while some other causes of death had a strong genetic, not environmental component, in this interesting study design).[331] The genetic vs. environmental (including diet, smoking, etc.) components in 15 cancers were estimated in Sweden (only because the specialized data needed is available in their Family-Cancer Database of over 10 million people). Environment was estimated to be the majority cause in each cancer type except thyroid (even for that cancer, just 53% estimated heritable-caused). Tumors at endocrine glands were the next highest--but just 28% were heritable-caused. The least heritable-cause ones were stomach cancers and leukemia: just1% of cases are estimated to be heritable (i.e. 99% environment-caused).[332]

A recent study illustrates well the influence of chemical exposure on chronic diseases, finding that the increase then decrease of Non-Hodgkins Lymphona incidence in Sweden tracked the ban 20 years ago of various toxic chemicals whose NHL cancer potency is well established. Similar NHL incidence trends have been noted in Finland, Denmark and the U.S, where many of these organochlorine pesticides and other chemicals have also been banned or restricted over the years[333] (for more details see our critique of 2,4-D's chronic tox. tests).

A comprehensive look at the known causes of cancer has also debunked Doll & Peto's estimate, using different approach. Gathering the whole world cancer mortality data (more reliably collected than is incidence data), they tasked expert groups with an exhustive and systematic review (including some raw data and some re-analysis) of all known cancer risk factors; to estimate, by data reliability, exposures and cancer potentcy. The nine environmental risk factors with reliable data (covering: infection, overweight, smoking, ethanol and certain air pollutants) are estimated to cause just 35% of worldwide cancer (deaths)--37% in the developed world.[334] Those few known-carcinogenic air pollutants are assigned to only 1% of all cancer deaths, so if we subtract them (making 34%), and add the 20% of cancers estimate to

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[327] K. Bridbord et al 15 Sep. 1978 'Estimate of the Fraction of Cancer in the U.S. Related to Occupational Factors'. NCI/NIOSH/OSHA, Bethesda MD.

[328] Interestingly, this much-replicated effect vanishes when the immigration occurs after adulthood (around 20 years old); indicating that insult & injury early in life is necessary for most cancers.

[329] P. Lichtenstein et al. 13 July 2000 'Env. & Heritable Factors in the Causation of Cancer' New England J. Medicine 343:2:78-85.

[330] Ntl. Cancer Institute 17 June 2004 'Benchmarks', Bethesda MD.

[331] Sorensen T. et al. (1988) 'Genetic and environmental influences on premature death in adult adoptees' New England Journal of Medicine:318:12:727-32 (24 March 1988).

[332] Czene K et al. 10 May 2002 'Environmental and heritable causes of cancer among 9.6 million individuals in the Swedish Family-Cancer Database' Int J Cancer:99(2):260-6.

[333] L. Hardell and M. Eriksson Nov. 2003 'Is the Decline of the Increasing Incidence of Non-Hodgkin Lymphoma in Sweden and Other Countries a Result of Cancer Preventive Measures?' Environmental Health Perspectives:111:14:1704-6.

[334] G. Danai et al. 19 Nov. 2005 ' Causes of Cancer in the World: comparative risk assessment of nine behavioral & env. risk factors' Lancet:366:1784-92.

be caused through inheritence (above); then about half (46%) of cancers are estimated to be caused by environmental factors other than the obvious ones of diet and smoking that the public health community typically associates with "environmental causes". **Thus it is intriguing that this careful analysis ends in the same estimate (a doubling) as does our informal derivation of the increase in USA cancers since the NCI's first national estimate in 1940! *Coincidence, or a proof that pollution is responsible for a doubling of cancer?! Either way, the evidence is overwhelming that cancer has doubled since the saturation of the biosphere with tocic chemicals. Prevenion, anyone?***
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INCIDENCE TRENDS -- OTHER CHRONIC DISEASE

Recently the World Health Organization (WHO) estimated that *one third* of child deaths in Europe are due to environmental causes (driven largely by Eastern Europe--pollution, sanitation, injury); not even counting mortality during conception and gestation, and only estimating clearly established causes of juvenile death and disability (e.g., only air pollution from the few well characterized air pollutants was considered--an estimated 13,796 deaths in in 0-4 year-old range).[335] Unlike adults, diet and smoking are almost nonexistant as causes of death in children, so that only injury competes with chemicals and similar environmental agents to kill children. Finally--and as always--chemicals certainly account for a greater portion of death and injury than that, since chemicals are much less studied than all other causes of disease--whether environmental or genetic causes.

Despite this, and the USA's failure to create registries of people with other chronic diseases, it is by now undeniable that a variety of other diseases are also strongly increasing or exploding in incidence here.

First, the only incidence data in the USA for non-cancer/non-birth defect chronic diseases are the ongoing, random, large and detailed national surveys by the Center for Disease Control & Prevention (CDC). From 1986 to 1996, the self-reported prevalence of disease categories tracked increased dramatically--over 20% for respiratory, endocrine/metabolic and nervous system diseases; with smaller growth for the self-reported skin and reproductive/fertility categories.[336]

Second, **a mechanism involved in almost all[337] chronic diseases is the activation of the *immune system* in response to foreign agents**--whether trauma, pathogens and chemical--in its immediate environment; typically the blood/air interface, the gut, the sensory nerves (which bypass the blood/brain barrier), or at the skin. Activation of the immune system is termed inflammation--the eponymous name for the symptoms of inflammation, observed since antiquity.[338] Inflammation is a knife-edge balancing act--acute and chronically--because its' tools to kill the foreign agent (strong oxidation, cell membrane-breaching, digesting molecules; and more) obviously can be just as hazardous to healthy tissue. Inflammation's stop and go signals are tremendously nuanced to achieve this knife-edge balance act required for a healthy organism.[339]

The well-intentioned attack of inflammation is already known to cause numerous chronic diseases, and to contribute to many more. **Auto-immune diseases** are clearly diagnosed in 5% of people in industrial countries, with additional victims and additional diseases obviously unrecognised.[340] In addition to obvious immune diseases such as respiratory syndromes (asthma, reactive airway diseases, etc.), and a panoply of undisputedly auto-immune, common (and seemingly more common) diseases such as type-1 (juvenile onset) diabetes, lupus and arthritis; inflammation also plays a definitive role in causing various cancers (but also in preventing cancerous cells from establishing themselves), in cardio-vascular diseases (including heart attacks and stroke), nervous system diseases, and even has a partial role in type 2

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[335] F. Valent et al. 19 June 2004 'Burden of Disease Attributable to Selected Environmental Factors and Injury AMong Chidren and Adolescents in Europe' Lancet:363:2032-9 (**also released** directly by the WHO by the same title and authors, in Environmental Burden of Disease No. 8, Geneva).

[336] Jill Litt et al. Oct. 2004 'Identifying Priority Health Conditions, Env. Data, and Infrastructure Needs:...' Env. Health Persp.:112:14:1414-8. Their graph of this is built from National Health Interview Survey (NHIS) data at http://www.cdc.gov/nchs.nihs.htm (accessed 13 July 2004).

[337] "[Inflamation] may well turn out to be the elusive holy grail of medicine, the single phenomenon that holds the key to sickness and health." - Dr. Wlm. Meggs 2003 'The Inflamation Cure', with Carol Svec; Contemporary Books.

[338] Around A.D. 40 Celsus observed it as "**rubor, calor, dolor et tumor**": irritation, heat, pain and swelling. Around the same time, Hippocrates said: **"Give me a fever, and I can cure any illness."** (i.e., fever denotes inflamation).

[339] U. Weiss (Ed.) 19 Dec. 2002 'Nature Insight: Inflamation' Nature:420:6917:845-889 (also reprinted as a supplement to the daughter-journals Nature Immunology and Nature Medicine)--**this is an excellent overview** at this time of this developing area of biology.

[340] D. Jacobson et al. 1997 'Epidemiology and Estimated Population Burden of Selected Autoimmune Diseases in the U.S.' Clinical Immunol. Immunopathol.:84:223-43.

("adult, non-autoimmune") diabetes.[341] A brief overview follows.

Even when **airway diseases** were named and categorized after their symptoms, it was apparant that many were similar in etiology (origin), as well as effect--in short, are essentially the same disease. That their origin lies in inflamation is proved not only by knowledge of their origin, but by anti-inflamatory drugs (both steroidal and non-steroidal) are used to treat them. **Asthma** has simply exploded in incidence in recent decades (current prevalence: 7.5% of American adults alone[342]). From 1980 to 1994 self/family reported asthma increased 75% in all Americans--but 160% among pre-school age children.[343] Physician-diagnosed asthma is 5-11% of children,[344] having increased in prevalence 58%, 1982 to 1992.[345] Although recently the asthma explosion has levelled-off in some (but not all) developed countries; part of this deceleration may be false: the increased use of effective therapies. The surveys from which prevalence rates are calculated are carefully done though they have biases, but blood samples over a long period in the UK found increasing numbers of citizens with atopy, rather than the suspected but untested decline in atopy over a lifetime.[346] Asthma adverse effects and deaths are increasing too, despite technologic advances. Very early changes in the learned immune system are implicated in asthma. Many studies confirm that asthma is triggered by environmental agents, including allergens and pollution.[347] **Chronic Obstructive Pulmonary Disease** (**COPD**), a broad respiratory disease category encompassing emphysema and chronic bronchitis, is estimated now to heavily impair 13.3 million Americans,[348] some 24 million Americans in total[349] (killing 120,000 a year, the 4th leading cause of death in the USA). Though much COPD is caused by smoking, its incidence has apparantly increased even as smoking rates were drastically dropping in the USA.[350] Asthma features more acute episodes of restricted airways, while COPD is more of an ongoing permanent airway restriction, as determined in part by differences in which pro-inflamatory molecules are present in either disease (more eosinophils in asthma).[351]

Multiple Chemical Sensitivity (**MCS**) is classic allergy where an initial exposure stimulates the immune system to create antibodies that, upon the next exposure to the agent, can begin the inflammatory reaction much sooner--except that the agent is a chemical, not a natural allergen. MCS' prevalence rate are in the USA has been confirmed several times at 12-15%, and it seems to affect all categories of people.[352]

Type 1 diabetes clearly involves inflamatory/autoimmune mechanism (attack on the insulin-producing cells of the pancreas), even if its ultimate causes are more involved. It has increased in incidence,[353] apparantly at 3-5% annually over the past years(!), a faster rate than any genetic change could cause.[354] While some have a genetic susceptibility to this disease, three independent studies found this susceptable genotype in increasingly fewer Type 1 patients, further implicating an environmental cause.[355] **Type 2 diabetes** (gradually increasing resistance to insulin) has a phenoomenally complex and largely environmental (not animal diet alone, and in part inflammatory) etiology . It is famously exploding in incidence, while Type 1--long known to involve an auto-immune attack on the cells that produce

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[341] Weiss 2003.

[342] Centers of Diseases Control & Prevention Feb 27 2004 'Asthma prevalence and control characteristics by race/ethnicity--United States, 2002' Morb Mortal Wkly Rep.:53(7):145-8.

[343] D Mannino et al. 1998 'Surveillance for Asthma Prevalence - United States, 1960-1995' Morb Mortal Wkly Rep.:47(SS-1):1-28.

[344] Lara Akinbami et al. 15 Jul. 2003 'US Childhood Asthma Prevalence:..' Amer. J. Epidemiology:158:2:99-109 (including a commentary exchange).

[345] N Clark et al. 1999 'Childhood Asthma' Env Health Perpsectives:107(3):421-9.

[346] M. Law et al. 21 May 2005 ‘Changes in atopy over a quarter of a century, based on cross sectional data at three time periods’ BMJ:330:1187-8.

[347] Gina Solomon 10 Apr. 2003 'Asthma & the Environment', a peer-reviewed summary of the data available at http://protectingourhealth.org/newscience/asthma/2003-04peerreviewasthma.htm .

[348] I. Rosenfeld 9 Nov. 2003 'Face the Facts About Lung Cancer' Parade Magazine, p. 10-12

[349] Lauran Neergaard 18 Nov. 2003 Associated Press story on COPD.

[350] E. Sutherland & R. Martin Nov. 2003 'Airway Inflamation in Chronic Obstructive Pulmonary Disease: Comparisons with Asthma' J. of Allergy & Clinical Immunology:112:819-27.

[351] Sutherland & Martin 2003.

[352] S. Caress & A. Steinemann Sept. 2003 'A Review of a Two-Phase Population Study of Multiple Chemcial Sensitivities' Env. Health Perspectives:111:12:1490-1497.

[353] P. Onkamao et al. 1999 'Worldwide Increase in Incidence of Type 1 Diabetes' Diabetologia:42:1395-403.

[354] Marian Rewers & P. Zimmet 6 Nov. 2004 'The Rising Tide of Childhood Type 1 Diabetes--what is the Elusive Environmental Trigger?' Lancet:364:1645-7.

[355] Rewers & Zimmet 2004.

insulin--has also grown. **Heart attacks and strokes** mostly evolve, and seem to be triggered by, inflammation; and though an animal and high-fat diet is an obviously-major cause for this tragic epidemic, inflammation from pollution is also a well-established cause.[356] A phenomenally complex syndrome in rich countries, **Metabolic Syndrome**, is coming into focus. It includes obesity, diabetes and cardiovascular disease (heart attacks and strokes) and high blood pressure; once again with inflamation (i.e. the environment) playing a key role.[357]

The **neurodegenerative** diseases, a broad category of chronic neurologic disorders that includes Alzheimer's, Parkinson's and Huntington's Diseases (AD, PD & HD), Amyotropic Lateral Sclerosis (ALS or 'Lou Gehrig's Disease'), Creutzfeldt-Jakob disease [CJD, the human version of the Transmissible Spongiform Encephalopathies--"Mad Cow", scrapie in sheep, Chronic Wasting Disease (CWD) in deer & elk, etc.] are all characterized by a build-up of protein plaque, by the degeneration of nerve cells, and apparantly by the presence of neurofibril protein tangles. Many plaques are amyloid, or waxy-like and perhaps non-nitrogenous (proteins contain nitrogen). Inflamation plays an important role in the neurodegenerative diseases, too;[358] as do metal/protein interaction and oxidative damage. AD is dominant: two thirds of all dementias; about 4.5 million Americans now, projected to be diagnosed in 13.2 million by 2050, a 3-fold rise from 2000 due to aging of a growing population alone.[359] Multiple sclerosis (MS) is degeneration of myelin (nerve insulation), not of nerve cells as are these other neurodegenrative diseases, but inflamation again plays a roll, this time interacting with the rebuilding of myelin. A key protein necessary formyelin production in glial cells (and for how much is produced) has finally been identified.[360]

While as usual, there are no registries for the incidence of neurodegenrative diseases; *a coherent and comprehensive analysis of neurodegenrative death rates across the industrialized world confirms that in developed nations they are both increasing in inicidence, and appearing earlier in life than they were 20 years ago (after adjusting for better diagnosis and increasing lifespans); thus toxins and diet (the increase was smaller in Japan) are implicated.*

PD especially is strongly associated with environmental causes, including pesticides and heavy metals. Worldwide PD incidence in 2003 was at least 17 per 100,000; while lifetime risk is a much as 4.4% for all PD like syndromes.[361] Synthetic heroin (MPTP), manganese, the fungicide maneb, the insecticide rotenone and the herbicide paraquat (among other pesticides) are all-but proven causes of PD. A huge study of twins found similar PD rates in identical and non-identical twins, strong evidence for environmental factors, at least when onset is beyond age 50.[362] However early (developmental) exposure in these and other neurodegenrative disease is alsmost certainly a key factor.[363] As with othr diseases, studies of incidence rates in the origin & destination countries of immigrants support environmental causes of PD; and (less convincingly) between industriliazed and undeveloped countries.[364]

AD too is an obvious disease, therefore its incidence time trend should not be influenced diagnostic breakthroughs. That its incidence has recently exploded only in developed countries[365] strongly implicates environmental factors as the main and increasing cause. .[366] A study of MS also shows it is strongly rising.[367] Environmental chemicals and lifestyles (e.g. diet) are among the causes of neurodegenerative disease being implicated.[368]

Metals in the environment are often toxic because they mimic the metals that biology uses to help shape delicate proteins.

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356 E.g. see the large experimental and epidemiologic literature establishing that fine particulate matter air pollutants (and other air pollution) causes cardiovascular disease.

357 S. Grundy 10 Dec. '03 'Inflamation, Hypertension and the Metabolic Syndrome' (Editorial) JAMA:290:22:3000-02.

358 Weiss 2002.

359 L Hebert et al. 2003 Aug. 'Alzheimer disease in the US population: prevalence estimates using the 2000 census' Arch Neurol:60(8):1119-22.

360 N. Seppa 10 Sept. 2005 ‘Critical for Coating’ Science News:168:11:163 (paper is in the 1 Sept. ’05 Neuron, one author is JL Salzer).

361 D. Twelves et al. 2003 ‘Systematic review of incidence studies of Parkinson's disease’ Mov Disord:18:19-31.

362 C. Tanner et al. 1999 ‘Parkinson disease in twins: an etiologic study’ JAMA:281:341-346.

363 P. Landrigan et al. Sept. 2005 ‘Early Environmental Origins of Neurodegenerative Disease in Later Life’ EHP:113:9:1234-8. This is the lead paper in a mini-monograph (7 papers) of a conference by the same title.

364 Landrigan 2005.

365 K. Ritchie & S. Lovestone 2002 'The Dementias' Lancet:360:1759-66.

366 C. Pritchard et al. June 2004 'Changing Patterns of Adult (45-74 years) Neurological Deaths in the Major Western World Countries 1979-1997' Public Health:118:4:268-83.

367 D Wynn et al 1990 'A Reappraisal of the Epidemiology of Multiple Sclerosis in Olmstead Co., MN' Neurology:40:780-6.

368 For a quick review of the evidence, see Sandra Steingraber 4 Sept. 2003 'Origins of Dementia, Part 2' Rachel's Env. & Health News #777 (avail. at http://rachel.org/bulletin/index.cfm?St=3)

Lead (Pb) exposure causes the physical degeneration and the behaviroal changes of AD in rodents, and it increases the level of the transcription factors known necessary to create the aberrant amyloid protein that is associated with AD (yet only when administered early in life!).[369] Extremely low levels of mercury (but not other metals) cause in controlled animal experiments the nerve cell changes observed in the brains of AD people; possibly because bivalent metals such as mercury have few sulfhydryl groups to bind to (and be sequestered) on the APO-Ee4 version of a protein that is more common in AD people than the other two variants of this protein.[370]

Other interfaces between the immune and neurologic system also show widespread inflammatory disease, although toxins also or concurrently affect the neurologic system directly.

Attention Deficit/Hyperactivity Disorder (**ADHD**) is a complex neurologic/cognitive syndrome, thus controversial with the usual lack of an incidence registry. For 2003, the CDC estimated in a national survey that 7.8% (4.4 million) of children 4--17 years old had ever had an ADHD diagnosis, in the middle of earlier estimates.[371] But is it *truly* increasing in incidence? There is no dispute its *diagnosis* has approximately trippled over the two or three decades--e.g. a brand new, still unpublished large survey of teachers found a prevalence of ADHD diagnosis of 7%, in line with similar findings.[372] It seems that the 1991 decision to extend additional federal aid to schools with diagnosed ADHD students would drive schools to falsely diagnose ADHD, as may the larger (more chaotic) class sizes over this period; changes in parenting are an unknown factor. Disorders such as ADHD involve failure of behavior that inhibits response--i.e. behavioral errors of commision. Although most diagnostic criteria are designed to detect ADHD in children, there is no doubt that ADHD is very prevalent in adults—one national survey conservatively estimates it is in 4.2% of workers, consistent with previous estimates.[373]

An important study finds not only that increasing prenatal polychlorinated biphenyl (PCBs) correlate with poor behavior inhibition later on, but that the PCB level correlated with decreasing splenium size--a part of the anterior corpus callosum involved in visual object recognition & discrimination, and in integrating observation w/ motor response; and which thyroid hormone develops neonataly (PCBs being famous thyroid hormone disputors).[374] That a few years lapsed between fetal PCB measurement the MRI scan is discounted as a confounder, because the size of the splenium is stable, at least from 4 yrs old on. Given the flooding of the planet with vast quantities PCBs, and the apparent explosive pandemic of ADHD and perhaps other behavioral disorders in young people that followed, this is an important finding, if independently replicated. Indirect support comes from an earlier study finding that two neuortoxins—PCBs and lead—each cause monkeys to perform as ADHD humans do in in behavioral and other tests (which are amazingly objective tests).[375]

The **autism spectrum disorders**, or 'pervasive developmental disorder' (here simply 'autism'), whose hallmark is failure of early (prior 3 years of age) social development, are a more discernable neurologic disease than ADHD. Autism has exploded in incidence/prevalence over the past 20 years, although diagnostic changes, overlap with other syndromes and heightened awareness (e.g. it began to be required to be reported in USA schools in 1991) have contributed to the increase. At least five published evaluations conclude that its prevalence has increased about ten-fold, from about 1 in 2,500 children in the mid-1980's to about 1 in 250 in the mid-1990's (40 per 10,000).[376] Similarly, whereas prevalance estimates in the past were consistant at 2 to 5 cases per 10,000; recent prevalence estimates are 60/10,000 or higher.[377] Such consistency in analysis by independent teams argues that the autism explosion is real, not caused by

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[369] NH Zawia Jul 15 2003 'Transcriptional involvement in neurotoxicity' (review) Toxicol Appl Pharmacol.:190(2):177-88.

[370] J Mutter et al. Oct. 2004 'Alzheimer Disease: Mercury as pathogenic factor and apolipoprotein E as a moderator' Neuroendocrinology Letters:25:5:275-83.

[371] Centers for Disease Control & Prevention 2 Sept. 2005 'Mental Health in the United States: Prevalence of Diagnosis and Medication Treatment for Attention-Deficit/Hyperactivity Disorder --- United States, 2003' MMWR Weekly:54:34

[372] 'Mental Illness in Children -- Part IV' by Vickie Que, reported on National Public Radio's Morning Edition news program, 25 Sept. '03. **See also:** S. Rowland 2002 'The epidemiology of attention-deficit/hyperactivity disorder (ADHD): a public health view' Ment Retard Dev Disabil Res Rev:8(3):162-70; **and** D. Connor 2002 Feb 'Preschool attention deficit hyperactivity disorder: a review of prevalence, diagnosis, neurobiology, and stimulant treatment' J Dev Behav Pediatr:23(1 Suppl):S1-9; **and** S. Dalsgaard 2001 Dec.'Reassessment of ADHD in a historical cohort of children treated with stimulants in the period 1969-1989' Eur Child Adolesc Psychiatry:10(4):230-9.

[373] RC Kessler et al. June 2005 'The Prevalence & Effects of Adult ADHD On Work Disorder In a Nationally Representative Sample of Workers' J Occup & Env Med:47:6:565-72.

[374] P. Stewart et al. Oct. 2003 'Prenatal PCB Exposure, the Corpus Callosum & Response Inhibition' Env Health Perspec:111:13:1670-7.

[375] Deborah Rice June 2000 'Parallels Between ADHD and Behavioral Deficits Produced By Neurotioxic Exposure in Monkeys' Env Health Perspect:108:suppl.3:405-8.

[376] D. Geier & M Geier March 2004 'A Comparative Evaluation of the Effects of MMR Immunization and Mercury Doses...' Medical Science Monitor:10:3:P133-9; see citaions 19-23.

[377] F. Volkmar & D. Pauls 4 Oct. 2003 'Autism' Lancet:362:1133-41.

diagnosis differences, migration patterns or other confounders. The increase is evident worldwide, including the UK, Quebec and Saudi Arabia.[378]

The state of California counts cases under treatment only, a very conservative method. Their Dpt. Of Developmental Services estimated that the core (best defined) autism disorders almost tripled in prevalence, 1987 through 1998.[379] This estimate was criticized by many in the medical community,[380] but the key criticsms--that parents of autistic children migrated to California seeking its perceived superior child care services; and that diagnostic criteria had eased--were refuted in the state's follow-up study; which also showed that their autism rate continued to accelerate slightly, from the end of the preceeding study through 2002.[381] Moreover, the California increase was independently confirmed; accounting for changes in the criteria to diagnose, the attraction of California's superior social services, and any overlap with mental retardation diagnosis. Not yet published, the study has been criticized for using too small a sample and failing to account for possible confounding by increased public awareness of autism.[382] But even after adopting yet stricter diagnostic citeria in 2003, autism incidence continued to explode! (rising 13%, 2003 to 2004).[383] Another prevalence study used Census Bureau data on births and state & federal education agencies' data to confirm that autism prevalence did indeed increase (the most for those born 1987-92, somewhat less strong growth after that).[384]

There appears to be consensus that impoved diagnosis does not account for the majority of the apparant increase in incidence.[385] That autism's diagonosis criteria haven't changed since 1994 also supports the this being a real worldwide epidemic. Epilepsy and brain changes are almost ubiquitous in autistic children, sealing the case that autism has physical, not social, roots (interesting but maybe coincidental, is that the brain grows in autism and shrinks in ADHD).

Autism runs strongly in familiess[386] but it does not appear that many families have more than one case; again it would be impossible for genetics to cause the apparnat exxplosion in incidence. Other evidence indicates that environmental agents influence the incidence of autism. For example, ~70% of autism strikes boys ...and males have less of the critical anti-oxidant glutathione (and all children have less, vs. adults). Sure enough, autism has been associated with low glutathione levels.[387] The brain is particularly vulnerable to oxidative damage, and that author followed up by finding that cultured human brain cells with less glutathione suffered more oxidative damage than those with sufficient glutathione; when mercury--a leading suspect in causing autism (it's used as a preservative, thimerosal, in vaccines)--was added.[388] Molecules such as glutathione are especially critical in detoxifying mercury because they have sulfur at their heart (as a thiol group). Mercury has a tremendous affinity for sulfur; thus glutathione normally scavenges toxic mercury.

In sum, consider the opinion of the Republican chairman of the U.S. House of Representatives committee investigating the causes of autism:

> "In the past, autism was considered a rare disease, affecting roughly 1 in 10,000 children," stated Chairman Burton. "Today, even conservative estimates of autism rates in the United States indicate that 1 in every 500 children is afflicted with the neurodevelopmental disorder, while other scientific studies reported in the Journal of American Medical Association have observed rates as high as 1 in every 150 children are affected, and the problem only continues to escalate."[389]

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[378] F. Edw. Yazbak 24 Jan. '04 (letter) 'Autism Seems To Be Increasing Worldwide, if Not In London' BMJ:328.

[379] Calif. Dpt. of Developmental Services 1 March 1999 'Changes in the Population of Persons With Autism and Pervasive Developmental Disorders in California's Developmental Services System', Sacramento CA; avail. at http://dds.ca.gov.

[380] E. Fombonne Feb. 2001 'Is There an Epidemic of Autism?' Pediatrics:107:2:411-2.

[381] U. Torassa 14 May 2003 'Autism Rate Doubles Amongst State's Kids' San Fransisco Chronicle; p. A-2.

[382] Erica Goode 26 Jan. 2004 'Autism Cases Up; Cause is Unclear' New York Times p. A-1.

[383] Katherie Seligman 4 Feb. 2005 'Scientists Baffled As Autism Cases Soar With No releif in Sight' S.F. Chronicle, pg. A-1.

[384] CJ Newschaffer et al. March 2005 'National Autism Prevalence Trends From United States Special Education Data' Pediatrics:113:3:e277-82.

[385] M. Blaxil 2004 'What's Going On? The Question of Time Trends In Autism' Public Health Reports:119:536-51; **and** L. Croen et al. 2003 'A Response to Blaxil, Baskin & Spitzer on Croen et al. (2002), 'The Changing Prevalence of Autism in California' ' J. Autism & Developmenta Disorders:33:2:227-9.

[386] Volkmar & Pauls 2003.

[387] Jill James et al. 2004 'Metabolic Biomarkers of Increased Oxidative Stress in and Methylation Capacity in Children with Autism' Amer J Clin Nutrition:80:6:1611-7.

[388] Jill James et al. 2005 'Thimerosal Neurotoxicity is Associated with Glutathione Depletion: protection with glutathione precursors' Neurotoxicology:26:1:1-8.

[389] Subcommittee on Wellness & Human Rights (http://www.reform.house.gov/WHR), Govt. Oversight Committee 19 Nov. 2003 Press Release.

Developmental disability in general is estimated to strike 17% (12 million) of U.S. children.[390] An expert panel estimated that a bare minimum of 3% of developmenta and neurologic deficits in children are caused by chemicals; 25% are caused by genes and chemicals interacting; and 5-20% are caused by environmental factors alone; a 'bare minimum' apportion because the data gaps on these effects of chemicals far outweigh what is known.[391] At least 4 million children have academic deficits, 20% of which have attentinal prolems.

Neurologic developmental toxcity is subtle yet devastating; causing many indirect social and economic problems. As David Rall, the former Director of the NIEHS (part of the NIH) said:

> "Suppose that thalidomide, instead of causing the birth of children with missing limbs, had instead reduced their intellectual potential by 10%. Would we be aware, even today, of its potential toxicity?"[392]

A 1% decrease in IQ (an imperfect measurement) across the USA would cost society at least $50 billion a year.[393] We spend some $200 billion a year on neurodevelopmental deficits, yet industry complains of the cost of regulating chemicals; even of testing (to find out what portion of those costs are caused by toxic chemicals). Such comparisons apply to all health effects, obviously.

Reproductive disorders have also clearly skyrocketed. More than half of all pregnacies fail (ignoring the high rate of pre-implantation failure, presumably). Similarly, pre-term and low birth weight births (risk factors for all manor of chronic disease) rates have increased significantly; but the role of pollution has not been well delinated. There is strong, not yet causitive evidence that pollution causes several reproductive failures involving sexual differentiaton (which is controlled by hormones and has been shown to be subject to hormone-disrupting chemicals): amazingly advanced puberty in girls; and a suite of exploding[394] male reproductive tract disorders (low sperm counts, malformed uretheras, undescended testicles, and testicular cancer), which may be controlled by an in utero 'testicular dysgenesis syndrome' of the testicle's master Leydig cells, which are easily disrupted by estrogens, including synthetic..[395]

In fact, the trend for disabling or serious **chronic diseases in children** may be increasing (in addition to the above evidence that childhood cancer rates have skyrocketed), though once again improving diagnostics and changing social definitions of disabilities complicate the determination. An older attempt to winnow these factors out concluded that either shifting perceptions or a true increase in acitivity-limiting chronic conditions had clearly occured from 1970 to 1981--from 2.6% to 3.6% of the population.[396] They evaluated and elimated five other possible causes for the increase: changes in the national survey, increased survival of low birth weight and the severely chronically ill infants, deinstitutionalization of ill children (conservitively, the survey doesn't sample the institutionalized) and better reach of medical services, especially to the poor. Current estimates of the trend indicate a *likely increase to this day*; though it's still problematic to measure. From 1982 to 2000, the proportion of child bed disability days attributed to chronic conditionsdoubled, 13% to 25%.[397] Similarly, the share of non-injury medical admissions attributed to chronic conditions was about 25% in 1960 but jumped to 55% in 2000.[398] Hospitalization rate and length of stay in Washington state fell more slowly for children with chronic illness than for those without it--by 2000 a quarter of bed-days were for children with mutiple hospital admissions, vs. 4% in 1962.[399] Estimates of the current prevalence of disabling (activity-limiting, or requiring special health care) are consistent at about 18% of the non-institutionalized child population, which doesn't include an unknown number of additional children who are be at risk but cannot be surveyed.[400] Non-trauma accounts for 80% of child medical

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390 Carolyn Raffenspurger 2003 'Precautionary Principle: bearing witness to and alleviating suffering' Rachel's Environment & Health News #761; http://erf.org.

391 National Academy of Sciences 2000 'Scientific Frontiers in the Development of Toxicology & Risk Assessment.' Wash. DC (pp. 19-20).

392 B. Weiss 1998 'Behavioral Neurotoxicity', quoting Dr. Rall. In: A. Lundberg (ed.) The Env. & Mental Health: a guide for clinicians (pp. 25-41) Hillsdale NJ: Erbaum.

393 B. Weiss 2000 'Vulnerability of Children and the Developing Brain to Neurotooxic Hazards' Env Health Perspectives:108(Suppl 3):375-81.

394 EWG 2005.

395 Sharpe & Skakkebaek 1993.

396 P. Newacheck et al. Feb. 1988 'Trends in Activity-Limiting Chronic Conditions Among Children' Amer. J. Public Health:76:2:178-84.

397 P. Wise Sep./Oct. 2004 'The Transformation of Child Health in the United States' Health Affairs:23:5:9-25.

398 Wise 2004.

399 J. Nett et al. 2002 'Trends in Pediatric Hospitalizations of Children in Washington State By INsurance and Chronic OCndition Status, 1991-1998' Arch. Ped. & Adolescent Med.:156:7:703-9.

400 P. Newacheck et al. July 1998 'AAn Epidemiologic Profile of Children With Special Health Care Needs' Pediatrics:102:1:117-23.

expense, and it's spent on just 20% of all children.[401]

Finally, **injury** and **infectious disease** today are very tiny contributors to extended morbidity or to mortality, compared to chronic diseases.[402] Moreover infectous disease has not dropped as far as was predicted by the implementation of basic public health measures,[403] which indicates that immune systems may be being suppressed by environmental agents (even excluding the significant effect of the AIDS-causing HIV (Human Immuno-deficiency Virus), which suppresses the immune response).

THE IMPLICATIONS

Cancer alone costs the country at least $180 billion a year in health care and lost economic activity[404]--about 2% of all economic activity (GDP: ~$10 trillion/yr.). The total direct plus indirect (e.g. lost income) costs of all diseases have been estimated to in 2002 be over $2.1 trillion a year--about 20% of all the money generated in the USA (i.e. GDP).[405]

A not-yet published estimate of the total (direct and indirect) **costs of toxic chemicals *alone*** to the people of Washington state is about 1.7% of the state's economic output; ranging from $4.4 to $5.7 billion a year.[406] Extrapolated on a population basis, that's roughly $150 billon a year, nationally--what politican would not love to save a large chunk of so much wasted money (as the newspaper story asks)?!

After cancers and heart disease, Altzheimer's and related dementias take the next greatest toll on the economy: $100 billion a year in medical cost alone, rising to as much as $700 b./yr. of medical cost by 2050.[407] The economic cost of all chronic disease is staggering.[408] For the known environmental component of *just four* major childhood diseases (see the citation), the USA's financial (only) cost has been painstakingly and with a very conservative methodology (e.g. ignoring consequence throughout life) estimated to be $55 billion/year, or 0.3% of total health care cost[409] (admtitedly the absolute cost (only) is inflated by our highly inefficient health care system). Neurodevelopmental deficits alone were estimated to cost the USA between $82.5 billion to $167b. a year, featuring unique costs such as special education but not including the costs of secondary disabilities.[410] Given the NAS estimate that environment causes at least 5-20% of developmental disability, environmental causes cost society at least $4.2 to $33.4 billion a year--never mind the cost of suffering.

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[401] A. Elixhauser et al. 2002 'Health Care for Children & Youth in the U.S.: 2001 annual report on access, ...' Ambul. Pediatrics:2:6:419-37.

[402] P. Newacheck & N. Halfon Apr. 1998 'Prevalence and Impact of DIsabling Chronic Conditions in Childhood' Amer. J. Public Health:88:4:610-7.

[403] R. Pinner et al. 1996 'Trends in Infectious Diseases Mortality in the U.S.' J. Amer. Med. Assoc. 275:3:189-193.

[404] Center for Diesease Control & Prevention 2001 'Cancer Registries: the foundation for cancer prevention & control' Atlanta GA; avail. at http://www.cdc.gov/nccpphp/bb_cancer_reg

[405] S. Clauser 2004 'Use of Cancer Performance Measures in Population Health: a macro-level survey' JNCI Monographs #33:142-54 (Table 1, derived from Ntl. Heart, Blood & Lung Institute 2002 Fact Book (Ch. 4, pg. 64), Ntl. Institutes of Health, Bethesda MD; avail. at http://www.nhlbi.nih.gov/about/02factbka.pdf (accessed Jan. 2005) (thar report estimated cancer's cost at nearly the same as our precceding citation--$189.5 billion/yr., instead of $180 b.

[406] Bob Condor 11 July 2005 'Exposure to toxins driving up healthcare costs -- Exposure to toxins costs us billions each year, study shows' Living Well [weekly column] Seattle Post-Intelligencer (reporting on research by Kate Davis of Antioch Accessed 18 July 2005 at: http://seattlepi.nwsource.com/health/231858_condor11.html

[407] M. Saleem Ismail, "Trials, Tribulations and Triumphs in Alzheimer's Disease: Where Are We Now and Where Are We Going?" presentation at the Ithaca College Gerontology Institute conference, "Meeting the Challenge of Dementia," May 29, 2003.

[408] No other country even comes close to the 15% of all economic activity (GDP) that we spend on health (though it is an inefficient system that tightly rations health). The absolute dollar amount is also large (because our economic output (gross domestic product, GDP) is so large and inflated). Expressed per capita, to compare to other countries, it is shocking.

[409] J. Landrigan et al. July 2002 'Environmental Pollutants & Disease in American Children: estimates of morbidity, mortality and costs for lead poisoning, asthma, cancer and developmental disabilities' Env. Health Perspectives:110:7:721-728.

[410] AAMR 2004.

Getting such invisible (slow-motion)[411] epidemics under control requires above all else that we understand their causes. If only we spent the one dollar per person-year that it would take to thoroughly monitor such chronic diseases,[412] we would truly know if the scattered and incomplete medical reports that indicate many other diseases are significantly increasing in incidence, are true. That money would also cover improvements in exposure tracking , to aid the assignation of causation--or to support denying causation, for that matter. Only 6% of 1,400 common toxic chemicals evaluated by the General Accounting Office (GAO) are being monitored in people by EPA or agencies within HHS, such as the CDC.[413]

However, since the mid-1990's a giant, prospective study of infants and children diseases (to follow 100,000 chidren, from conception to 18 years) has been getting underway, the National Children's Study.[414] The NCS is the logical outcome of the recent awareness of the criticality of a healthy development to the entire lifetime of an organism. Thorough measurement of the mother's and the children's environment and bodies, and many state of the art tests of effect, are planned. As cause must preceed effect, it is more valid to test a cause & effect hypothesis prospectively rather than retrospectively. Combined with the size and thoroughness of the study, it will undoubtedly result in very reliable and interesting results. Other countries are also doing comprehensive studies of the impact of environement on human children and infants, though not every researcher is taking the time and trouble to formulate their hypothesis a priori, i.e. before they begin their tests (there is some logic to doing the environmental and body burden measurements first, and using the results to formulate hypothesis to then test).

In confirming that the NIH (the major source of medical research funding in the USA) allocates its disease-specific research money largely in proportion to the burden of those diseases, it was revealed that research on in-utero & infant diseases is not only severely underfunded (relative to other disease categories), but that it results in the highest years of lost life.[415] That is important because the period from conception to adulthood is the period most vulnerable to environmental insult (or inheritted insult, for that matter).

All the failures of chronic toxicity tests we have critiqued from the start of this paper are corroborated by the simple fact that proper chronic toxicity testing is very scarce in the medical and toxicologic independent, peer-reviewed literature.

The newly massive and inter-locked "life science"--chemical, bio-tech and harmaceutical--corporations, having succeeded in foisting the disaster of Q-RA on the people's government, now attempt to corrupt the very temple of society: academia. These private interests care nothing about destroying the very fabric of society.[416] They aim to pervert not only private universities; but even the people's heritage in the USA, the land-grant college! Well over 90% of life science companies report financial relations with universities, and the number directly supporting academic research increased during 1984 to 1994 from 46% to 57%[417] A critical early achievement by capitalism was passage of the bipartisan Bayh-Dole Act in 1981, which provided academia the lure of profit (lucre) in exchange for its knowledge (via licensing agreements); thus binding academia's public interests to the private interests of corporations--to privatize knowledge (turn it into property). But in the long run this perversion will fail, because knowledge is only created and enhanced when it is free. Science itself dies without full transperancy. Meanwhile, citizens are paying at least twice for knowledge: for its creation with taxes and for using it as consumers, via the monopoly rents of intelectual property;[418] yet they typically pay a third time: with their health. Sometimes we even pay a fourth time: when the same 'life-science' company sells both the disease agent, and its cure!

If it weren't for the scientific curiosity of individuals in academia--still largely free of the CoI of the corporations we allow to perform almost all tests for Q-RA, we would have almost no independent, peer-reviewed studies to begin to illuminate the toxiicty of environemntal eagents, nor the massive toxicity data-gaps. For the non-human portion of the biosphere (though it is actually a seamless biosphere), our ignorance is worse (e.g., see the critique of individual pesticide

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411 "Like a nightmare carved in molasses." -?

412 Johns Hopkins Sept. 2000.

413 GAO May 2000 'Toxic Chemicals: long-term coordinated strategy needed to measure exposures in humans', GAO/HEHS-00-80, Wash, D.C.

414 Jocelyn Kaiser 11 Jul '03 'Everything you Wanted to Know About Children, For $2.7 Billion' Science:301:162-3, is a good description of what is being planned for the NCS.

415 C. Gross et al. 17 June 1999 'The Relation Between Funding By the NIH and the Burden of Disease' NEJM:340:24:1881-7.

416 E. Press & J. Washburn March 2000 'The Kept University', The Atlantic Monthly p. 39-54; **and**: D. Shenk 22 March 1999 'Money + Science = Ethics Problems on Campus', The Nation p. 11-18; both thorough investigative stories.

417 D. Blumenthal et al 8 Feb. 1999 'Relationships Between Academic Insitutions & Industry in the Life Sciences--an industry survey' NEJM:334:368-73).

418 Drahos & Braithwaite 2004, at p. 26-7.

registrations, below). Finally, society assumes that our biosphere has an almost endless assimilative capacity, the stupidty of that assumption has been proven time after time--man depends completely on his biosphere.

Once again, pesticides are the only category of non-therapeutic (e.g. chemotherapy) chemicals-in-commerce *designed* to be poisons. So it is perhaps not surprising that the independent, peer-reviewed literature that does exist on them shows widespread risks to both human health and to ecosystems. *Risk assessors, chemical manufacturers & users and risk managers--such as the USFS--can cite no independent peer-reviewed data to support their claims of safety*. In fact, as we show exhaustively for many herbicides below, the independent scientific literature that does exist overwhelmingly supports our claims, as it does for any category of chemicals. Given the delicacy of biology, that is to be expected. Indeed, if we had the time, we believe we could provide a hundred times the number of peer-reviewed citations regarding the impacts of herbicides than we have, given that we have not thoroughly searched the literature for more than a handful of the herbicides, or listed hardly any of the many ecological effects of any herbicides.

HERBICIDE RESISTANCE & INTEGRATED WEED MANAGEMENT (IWM), AS TO THESE EIS'

When a precautionary approach is implemented, real questions of alternatives--and the relative net cost or benefit of these alternatives--do not disappear. Here, pesticides are ultimately self-defeating.

The FEIS fails to even mention a very large negative ecological impact of herbicide use--the inevitable, observed and continuing exponential growth in the resistance of weeds to herbicides. Evolution through selection presures--such as herbicides--is omnipresent in the biosphere, but the more focused and intense it is (e.g. man's pest management with a single tool, say, herbicide) that occurs, the greater the amount of natural selection that will adapt to this pressure (the survival of the fittest) to make that tool ineffective. Competing populations almost always achieve stability over time, in sometimes amazing ways.[419] The more rapid an organism's reproductive cycle (e.g. the approximately 10-20 minutes microbes take to reproduce) the faster it will develop resistance.

Often pesticide proponents argue that even though the development of reisstance is inneviatable, that development confers an evolutionary cost, and thus switching to a different tool will cause the resistance pest to die out, as natural selection again occurs. A paper shows this to be a stupid assumption (i.e. it went untested for too long), finding that insects that develop DDT-resistance pass on an *additional* genetic advantage to their offspring (via their mothers, possibly in a metabolic enzyme).[420] This data wipes out another stupid assumption, that alternating pesticides ovr time will significantly reduce resistance. That theory relies entirely on the previous assumption (that species gain resistance only by losing another competetive advantage). Alternating pesticides in space requires that the target species aren't able to migrate (e.g. by seed dispersal), but everyone agrees that happens all the time. Only applying a mixture of two or more pesticides co-temporaneously is known to significantly delay resistance,[421] but aparantly no-one has investigated the invevitable development of much more robust and powerful resistance from such a reckless challenge (and who knows what unanticipated effects arise such as synergistic toxicity arise from applying mixtures--without even partularly looking, and though it is not required to be tested; we have come across dozens of examples of synnergistic toxcity for herbicide active ingredients alone) Similarly the strategy of saturation is recklessly stupid. Only moderation has a logical underpinning in fighting the development of resistance. Natural selection is an especially powerful force when reproductio cycles are short, which is the case in most of the species that humans call 'pest'.

Available data shows that pesticide resistance is climbing exponentially across at least several of the basic kingdoms of life, even with an acknowledged lack of searching for cases of resistant species.[422] As to weeds, worldwide, at least 216 herbicide-resistant weed species were documented by 1999.[423] For example the herbicides of the picloram family, heavily relied on in this FEIS, are known to be resisted by some weeds.[424] At least one common western-United States weed family--spurge--has been proven to block picloram and 2,4-D herbicides from translocating to the root (where those herbicides kill the plant), explaining spurge's strong resistance to these herbicides.[425] 4 to 5 more weed species were added to the list of glyphosate-resistant weeds.[426] In fact, at least 15 weeds have naturally reistant strains or have developed resistance to glyphoste formulations.[427] Monsanto's solution to this--recommending the application of other broad-spectrum herbicides such as 2,4-D--will only accelerate the growth of resistance, by greatly increasing selection pressure.

Monoculture rice fields were treated with sulfonylurea herbicide-based mixtures for 8 consecutive years. The resistant type of M. vaginalis showed high levels of cross-resistance to pyrazosulftiron-ethyl, bensulfuronmethyl, cyclosulfamuron, and flumetsulam, but not to imazaquin; and did not show multiple resistance to other herbicides having different modes of action, such as simazine, propanil, oxadiazon, butachlor, and 2,4-D. In vitro and in vivo ALS assay results showed that

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[419] D. Pimentel 29 March 1968 'Population Regulation & Genetic Feedback' Science: 159:1432-7. This 35 year-old paper is an excellent introduction to the fascinating theory and observations of resistance, which Dr. Pimentel terms 'genetic feedback'.

[420] McCart C, Buckling A, Ffrench-Constant RH. 9 Aug 2005 'DDT resistance in flies carries no cost' Curr Biol.:15(15):R587-9.

[421] GS Mani April 1985 'Evolution of Resistance in the Presence of Two Insectivides' Genetics:109:761-83.

[422] P. Weber May-June 1992 'A Place for Pesticides?' WorldWatch Institute:22-23.

[423] S. Barber 1999. 'Transgenic plants: field testing and commercializadon including a consideration of novel herbicide resistant oilseed rape (Brassica napust)'; In: Lutman, P.J. 1999, Gene Flow and Agriculture: Relevance for Transgenic Crops. British Crop Protection Council. Farnham, Surrey, UK. 286 pp., pp. 311.

[424] E.g. Fuerst et al. 1996 'Physiological Characterization of Picloram Resistance in Yellow Starthistle' Pest. Biochem. Physiol. 56:149-161.

[425] Rodney Lym, Weed Science professor, N. Dakota State U., personal communication to Tony Tweedale, Sep. '01.

[426] Bob Hartzler 2003 'Are Roundup Ready Weeds in Your Future II' (extension weed management specialist, Dpt. of Agronomy, Iowa State U.) Avail at: http://www.weeds.iastate.edu/mgmt/2003/glyresistance.shtml

[427] VK Nandula et al. Aug. '05 'Glyphosate Resistant Weeds--current status & future outlook' Outlooks on Pest Management:16:4:183-7.

the resistance mechanism of M. vaginalis might be due to the altered acetolactate synthase.[428]

The diversity of 2,4-D-degradative plasmids in the microbial community of an agricultural soil was examined by complementation. By using agricultural soil that had been treated with 2,4-D for several years, transconjugants were obtained with both recipients, but not in the untreated control soil. The various transconjugants had plasmids with high degrees of homology to the tfdA gene. These results indicate that this soil microbial community submitted to selective pressure (application of 2,4-D) for several years maintained a diversity of self-transferable plasmids carrying diverse genes encoding 2,4-D degradation.[429] Yeast metabolism rapidly adapts to 2,4-D.[430]

A recent study examined the role of auxin-binding protein (ABP) in auxin herbicide resistance. There were no differences in uptake, transport, and metabolism of auxinic herbicides between the Resistant (R) and Susceptible (S) biotypes of wild mustard weed. Based on that, and on studies on the role of auxin-enhanced ethene biosynthesis and calcium in mediating the auxinic herbicide resistance, they hypothesized that resistance is due to an altered target site, possibly an auxin receptor. A small ABP gene family from both R and S types was characterized--amino acid changes were found in the ABP of the R biotype. The role of ABP in mediating auxinic herbicide resistance is still unknown.[431]

There are more plant species resistant to ALS-inhibiting herbicides (sulfonureas and imadazolinones) than any other family of herbicides, and more plant species are resistant to multiple ALS-inhibitors...despite these herbicides being much newer than almost all others.[432] This astounding fact is perhaps not suprising when you consider that ALS inhibtors are so toxic to all plants at extraordiarilly low doses (inhibiting the enzyme the need to produce three amino acids) that plants face no choice other than to immediately die or show a genetic diference that means survival (and usually thriving survival as its genetic competition has been killed). In short this is a classic example of selection pressure at work, illustrating the inevitability of significant resistance. Mare's Tail (horseweed) is currently resistant to glyphosate in 11 states, and its resistance seems to be exploding.[433]

The Weed Science Society of America has so far confirmed 250 cases of weed resistance to herbicides[434], and confirmed that known cases of herbicide resistance continue to climb exponentially, In Montana they report that there are currently 9 reported types of resistant weeds. They say that local (Montana) weed scientists estimate that there are 6,640 sites and more than 617,100 acres infested with herbicide resistant weeds in Montana, which infest barley, cereals, cropland, railways, sugar beet, and wheat.[435] Herbicide resistance by weed species on public lands is under-reported, given the higher economic value of cropland promotes interest in resistance.

Such data corroborate what is possibly the best proof that pesticides aren't working: conventional (i.e. pesticide treated) crop losses have never decreased, even after decades of intensive pesticide applications (12% of crops are still lost to weeds, 13% to insects and 12% to disease).[436] This fundamental fact is supported by at least one controlled experiment that showed four corn pests (3 insects and smut--a fungus) increased only following application of the popular corn herbicide 2,4-D.[437] Agriculture-dominated economies such as Indonesia's; and those of several developed countries in Europe have reduced pesticide use by more than half, to date, with no detrimental economic effect[438] (and that's on average, i.e. some have already achieved greater reduction of pesticide use). Finally, herbicide use on the scale of public

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[428] Hwang IT, Lee KH, Park SH, Lee BH, Hong KS, Han SS, Cho KY. 2001 Oct. Resistance to acetolactate synthase inhibitors in a biotype of monochoria vaginalis discovered in korea. Pesticide Biochemistry & Physiology 71:69-76.

[429] Top EM, Holben WE, Forney LJ. 1995 May. Characterization of diverse 2,4-dichlorophenoxyacetic acid-degradative plasmids isolated from soil by complementation. Applied & Environmental Microbiology 61:1691-1698.

[430] A Fernandes et al. 2003 'Activation and significance of vacuolar H+-ATPase in Saccharomyces cerevisiae adaptation and resistance to the herbicide 2,4-dichlorophenoxyacetic acid' Biochem Biophys Res Commun:312:1317-1324.

[431] Zheng HG, Hall JC. 2001 Mar-2001 Apr 30. Understanding auxinic herbicide resistance in wild mustard: physiological, biochemical, and molecular genetic approaches. Weed Science 49:276-281.

[432] Weed Science Soc. America 2003 'Herbicide Resistant Weeds Summary Table, avail at http://weedscience.org/summary/MOASsummary.asp **and** A. Hashem & H.S. Dhammu 2002 'Cross-resistance to Imidazolinone Herbicdes in Chlorsulfuron-Resistant R. Raphanistrum' Pest. Mangmnt. Sci.:58:917-9.

[433] Juliana Barbassa Aug. 10 2005 ' Associated Press 'Herbicide-resistant weed becoming a pest in California'.

[434] Regina Baucom & R Mauricio 'Fitness Costs & Benefits of Novel Herbicide Tolerance In a Noxious Weed' PNAS'101:36:13386-90.

[435] I. Heap, The International Survey of Herbicide Resistant Weeds. Online. Internet. November 25 2002. Available at http://www.weedscience.com

[436] Dr. David Pimental (Cornell U.), videotaped at Beyond Pesticides/NCAMP 2001 Conference, Boulder CO (available from BP/NCAMP).

[437] Pimental videotape (though also published in a scientific journal, the citation is unknown to us).

[438] Pimental videotape.

lands is expensive, even if we erroneously assume that they work in the long run. About the only known logical and efficacious use of herbicide is when an immediate and short-term effect is desired (such as to establish another vegetation species)--but even that can be problematic.[439] Some Roundup formulations induce the growth of dangerous and expensive plant molds such as fusarium (perhaps through a resistance mechanism), even at regular application rates (yet Monsanto is still pushing RoundUp Ready wheat, which it acknowledges will increase glyphosate use). This soil-organism effect may be due to the reduction of plowing that herbicides are used for; or due to an ingredient other than glyphosate, as different Roundup formulations have different effects.[440]

In contrast to the direct-attack approach that pesticides represent, integrated pest management (IPM, or IWM in the case of weed pests) uses *both* pest prevention and a detailed knowledge of a pest's life cycle and environment to effectively manage the problem, with greatly reduced pesticide use. A holistic and preventative solution to any problem in nature is intrinsically more likely to succeed than is a narrow and reactionary one such as chemical attack. The prevention of the introduction of seeds is the universally acknowledged single effective answer to weed spread, so it is not logical to attempt other weed control methods before seeing if prevention is effective.

Yet this FEIS attempts the opposite. Road construction is the undisputed major vector for the introduction of seeds into USFS lands, thus road construction and road use are natural focal points for weed prevention. In this FEIS only 160 of 16,000--or 1%--of the weed-infested acres planned to be managed every year will use alternatives to herbicides, most to be broadcast from the air.[441] An integrated and varied management approach is inherently more challenging to weeds and their ability to evolve resistance. Spraying 99% of the acres seems, on the basis of evidence presented here, highly unlikely to succeed in controlling weeds before they become heavily established, which is the stated fundamental purpose of this FEIS' evaluated action.[442]

It is worth questioning even the fundamental view of nature that we always take, here the ridiculously simplistic alleged distinction between weeds and native plants. For example, using a very large databases of plant species it was recently found that these alleged "weeds" are very much (three times) more likely to appear in the pharmacopoeia of highland Chiapas Mexico natives than would be predicted by the frequency of weed species there in general.[443] Obviously, disturbed, weedy areas are closer to humans than are many areas rich in native plants (such as the tropical forests around this human community), especially when you consider the short life-span of many pharmaceutical chemicals in plants. But the lead author adds:

> "There is also good biochemical evidence that supports the hypothesis that plants in disturbed areas are likely to have more chemicals in them than for defense."[444]

It is almost inevitable that such a strong preference ($p > 0.0001$!) to aquire drugs from weeds, not native plants, requires more of an explanation of convenience alone.

PESTICIDE REGISTRATION IS **NEVER** A WARRANTY OF SAFETY

EPA registration in *no way* warranties the human or ecological safety of a pesticide (as many defendants in tort actions continue to discover).

First and most important, registration is not designed to--and does not--guarantee a pesticide's safety. Under the USA's federalized pesticide law (FIFRA, the Federal Fungicide, Insecticide and Rodenticide Act), to distribute or sell an agent

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[439] See, e.g., our discussion of problem-causing residues of picloram-family herbicides in compost and in crop rotation, found at the end of our critique of pesticide registration.

[440] Adrian Ewins July 10 2003 'Scientists eye glyphosate-fusarium link' The Producer, Saskatoon Canada http://www.producer.com/articles/20030703/production/20030703prod02.html (the experiments were performed by USDA/ARS (at U. MO) and by Agriculture Canada's Swift Current research center at Saskatoon).

[441] FEIS Sec. 1.3, p. 1-3; and Table 2.4.2, p. 2-7.

[442] FEIS pg. ROD-1.

[443] J. Stepp & D. Moerman 2001 'The Importance of Weeds in Ethnopharmacology' J. Ethnopharmacology:75:1:19-23.

[444] In a 15 March 2001 U. of GA Press Release.

with pesticidal activity the agent must first undergo registration, i.e. receive a license.[445] As ammended, to allow registration (sale or use) of a pesticidal agent, the U.S. EPA must determine that its composition supports the pesticidal claims made by the registrant; that it will perform its intended function, that the proposed labeling and other submitted materials comply with FIFRA's requirements of registration; and finally that there will be no "unreasonable adverse risk to man or the environment, taking into account the economic, social and environmental costs and benefits of the use", and the risk of its residue in foods.[446] In sum, a pesticide is allowed on the market when EPA judges there is a net benefit to its use, after subtracting the known/estimated costs and risks from the known/estimated benefits. Note that basically only the “pesticidal”, i.e. the ‘active’ ingredient/s (a.i.) in a pesticide formulation is subject to this pre-market approval.

This “no unreasonable risk” standard that determines pesticide regulation is overwhelmingly a cost/benefit paradigm, not a public health one; and to a degree unlike any other major federal environmental statute. The direct benefit (to the manufacturer) of the sale of the pesticide is an enormous and easily qunatified benefit that by law must be considered. EPA almost never considers the benefit of alternative pest management systems, despite alternatives being effective[447] (and despite pesticides not being effective, as documented above). Importantly, the costs of pesticide use are far more diffuse than their benefits, and therefore are more poorly quantified or even understood; than are the benefits (this is a major flaw of all cost/benefit analysis tools[448]). EPA makes almost no attempt to correct these critical biases when registering these designed poisons under their “no unreasonable risk” mandate.

Second, FIFRA statutorally disallows even conditional claims that pesticides are safe. EPA has gone to considerable length to make people aware that registration does not warrant safety, or generally shield parties from tort liability. The numerous direct claims of herbicide safety that this FEIS makes[449] are illegal. FIFRA forbids using the fact that a pesticide is registered to claim that it is safe, even if that claim is modified (as in: “safe when used as directed”)[450] (in the context of commercial advertisement claims of safety , the FTC has ruled accordingly). Nevertheless, these claims are rampant and endemic.[451] They are in part the inevitable result of registering poisons for use; but in part the claims are rampant because EPA has again dropped the ball--it has no effective way to even determine if pesticide manufacturers, sellers and users are making these claims; much less a coherent program to stop them.[452]

Third, we will next show beyond any reasonable doubt--and contrary to these FEIS’ constant claims of the thoroughness of ecologic & human safety tests during registration[453]--FIFRA and its registration regulations are so inadequate a review of safety that an uninformed person will be stunned (we show this not only in this major section, but in our sections that criticize the specific Q-RA the USFS relies on).

The first *a priori* proof that FIFRA is no warranty of safety is that EPA doesn’t now (2003) project finishing even initial re-registration of the many original registrations (many decades out of date) until past 2030, long after the new renewals will have begun piling up. After FIFRA first became a comprehensive licensing law in 1972, about 50,000 pesticide products existed. Fourteen years later (1986) EPA had not under the stricter requirements re-registered a single one, nor had it completed re-assessing any of the pre-1972 a.i.![454] Only due to the disclosure of the massive testing fraud described below, Congress in 1988 mandated a specific schedule for achieving all this re-registration using the more rigorous standards.

Yet EPA, due mostly to political funding constraints, has fallen far behind its statutory re-registration deadlines; to date

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[445] FIFRA sec's. 3(a) and 12(a)(1).

[446] FIFRA sec. 3(c)(5), defined at FIFRA sec. 2(bb) (codified, 7 USC 136(bb).

[447] U.S. General Accounting Office Dec. 1991 ‘Better Data can Improve the Assessment of EPA’s Benefit Assessments’ RCED-92-32, Wash. D.C.

[448] Heinzerling & Ackerman 2002 ‘Pricing the Priceless: cost benefit analysis of environmental protection’ Georgetown [Univ.’s] Env. Law & Policy Institute, Wash. D.C. (for example).

[449] E.g. FEIS p. 2-3, 3-5, and throughout ch. 4.

[450] FIFRA 12(a)(1), implemented at 40 CFR 168.22.

[451] GAO 1990 ‘Lawn Care Pesticide Risks and Prohibited Safety Claims’ RCED-90-134 Wash. DC.

[452] GAO 1990.

[453] FEIS p. 2-3, 3-5, and throughout ch. 4.

[454] General Accounting Office 1986 'EPA's Formidable Task to Assess & Regulate Their Risks'.

having re-registered less than a third of all pesticides.[455] In the next major revision to FIFRA, the 1996 Food Quality Protection Act (which followed a smaller but still major pesticide testing scandal, Craven Labs--see below), Congress appeared to recognize that it was refusing to give EPA the budget to complete these re-registrations in a timely manner, and so required that EPA adopt the *goal* that all registrations be reviewed every 15 years, and clarified EPA's authority to demand any data it felt was needed to keep pesticide registrations permanently consonant with the current state of science.[456] In short, EPA is given the necessary specific authority it needs to determine "no unreasonable risk..", but not the means. FIFRA's historic trend is continued: very slow improvements in the use of the scientific literature in registration, but never even approaching a fair review, as we will show. In sum, most pesticides today are still used without even the minor assurances of safety that re-registration provides.

A second *a priori* proof is that even when EPA registers a pesticide today (i.e. when it attempts to determine “no unreasonable risk”), it fails regularly (funding constraints by politicians on EPA are again very likely responsible--but this time only in part). It is true that the long history of FIFRA and its predecessor pesticide law is a history of a constant tightening of safety requirements, since at least 1948. Yet today at least seven (three herbicide, four other) of even the most modern pesticide re-registrations have been shown to fail to determine the mandatory 'no unreasonable risk' determination.[457]

New-chemical pesticide registrations (i.e, not re-registering outdated registrations) of today are similarly defective. Of the new-pesticide registrations made 1997 to 2001 that did not receive waivers of test requirements (19 of 41), more than half (of the 19) were not fully tested anyway! That fact alone makes registration as a warranty of safety a joke--repeatedly violations of the fundamental mandate to determine “no unreasonable risk” (and a third *a priori* proof that registration is no warranty of safety).

But there’s more. Of the full set of 41 new pesticide chemical registrations (all of which supposedly passed the basic “no unreasonable risk” registration requirement, even though most received test waivers, in part for economic reasons) 20% were carcinogens, over a third were mutagenic (damaging DNA), almost 75% caused birth defects or reproductive problems and over 20% were neurotoxic! Almost one fourth had avian toxicity, almost half were moderately or highly toxic to fish on an acute dose basis, and almost 20% had a chemical structure that makes them likely water contaminants.[458] Of 750 pesticides registered currently, just nine have been tested on the developing nervous system, yet six of those nine were more harmful to young than to adult test animals.[459]

Similarly; whether a new, a re-registration, or an old not re-registered pesticide; registration does not even assure protection from deadly effects such as cancer and reproduction. Of the 26 most widely used pesticides in the USA, 12, with estimated usage of 380 million pounds of a.i. annually, are classified as carcinogens by the EPA (four have many citations in the peer-reviewed literature that they are carcinogenic in humans). Almost all these 26 very heavily used pesticides cause reproductive effects (nine cause male reproductive effects and 17 cause male reproductive or gestational effects), accounting for an estimated 900 million pounds of a.i. per year and estimated 700 million residential applications a year (residences are very vulnerable locations: intimate exposures and the likelihood of vulnerable (developing) children to be exposed--more heavily yet due to their crawling and hand-to-mouth behaviors).[460] An earlier Congressional Committee determined that in the 1,200 a.i. at the time, 79-84% of pesticide carcinogenicity data and 90-93% of the mutagenicity data were inadequate.[461]

The National Academies of Sciences (NAS) investigated the carcinogenic potency of 29 registered yet known carcinogen pesticides; they calculated that exposure to these pesticides *at allowable levels* would result in 20,800 cancers and about 10,000 deaths a year! (they identified a total of 53 carcinogenic pesticides, but 24 did not have sufficient data to estimate

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[455] Caroline Cox Apr. 2002 ‘Pesticide Registration: no guarantee of safety’ (http://www.pesticide.org/factsheets.html). This is an updated version of the same paper published: Cox Summer 1997 J. of Pesticide Reform:17:2:2-9 (journal of Northwest Coalition for Alternatives to Pesticides (NCAP) Eugene OR; a journal that publishes the results of peer-reviewed primary research (i.e. it does secondary research) in a thorough way, by citing every claim that it makes to peer-reviewed journals or to government research.

[456] U.S. EPA 2000 Advance Notice of Proposed RuleMaking, OPP DOcket #36195, published in F.R. ~ 26 April 2000.

[457] Cox 2002.

[458] NCAP Summer 1999 ‘Does Government Registration Mean Pesticides are Safe?’ J. Pesticide Reform 19:2:3. This survey was performed by NCAP. It is based on EPA’s registration factsheets that summarize all the data of new registrations. Also Cox 2002, p. 4.

[459] EPA/FIFRA Science Advisory Panel meeting 25-27 June 2002 'Determination of Appropriate FQPA Safety Factors in Organophosphate Pesticide Cumulative Risk Assessment' www.epa.gov/scipoly/sap2002/june/final_10x_cra.pdf

[460] NCAP Summer 1999 ‘Are Pesticides Hazardous to Our Health?’ J. Pesticide Reform 19:2:4. This excellent analysis makes 54 citations to independent scientific literature to prove these health effects of these widely used pesticides, and for the estimated annual numbers of applications and amounts applied.

[461] M. Coye 1985, J. Public Health Policy:6:349.

their cancer potency).[462]

Even 25 years ago the independent published literature on the carcinogenicty of registered pesticides was large and overwhelmingly persuasive (as opposed to the pesticide manufacturer's literature, whether published or not)--for example, a 1981 study correlating farming in Iowa with cancer mortality discusses a lot of old literature that supports their findings.[463]

A fourth *a priori* proof of the failure of the “no unreasonable risk” registration standard is that EPA; having fallen so far behind in re-registrations, and mandated to consider the impacts on a manufacturer of restricting a pesticide’s use or of banning it; happens to be issuing many re-registrations that require future risk tests, to fill data gaps in the tests performed for registration.[464] EPA is tacitly admitting that it has not made the basic determination of “no unreasonable risk” before registering (allowing) use. Calling for study appears to serve the same purpose as do EPA’s warnings that registration is no warranty of safety. Both give warning to potential tort defendants. More fundamentally, they facilitate the registration of dangerous pesticides that should not be registered under the “no unreasonable risk” standard, to the great benefit of the registrant. Even worse, such techniques serve to hide the flaws of registration, both regulatory (see below) and as practiced (see the above examples). How can EPA possibly determine there is no unreasonable risk if it has to call for further study?. Evidently, it is instead a technique to get highly risky--perhaps unreasonably so--pesticides registered for sale. Certainly there is tremendous pressure in cost/benefit balance of registration to consider the economic benefits of pesticide sales.

A final *a priori* proof of the failure of FIFRA to warranty safety in any manner comes from a compelling natural (i.e. unplanned) experiment. Many fungicides are also anti-fungal drugs, so they are tested by both FIFRA’s and the FDA’s safety protocols. Of the top three selling fungicides, the one that was also used as an anti-fungal drug showed up in over 8,000 citations in Medline (an interface to the National Library of Mecicine’s world-leading indexes of the independent scientific literature). The two fungicides that had only undergone FIFRA’s safety protocols showed up in less than 20 Medline citations each![465] Pesticides are supposed to be far less critical to society than drugs (e.g., we allow toxic drugs on the market, if needed), thus their saftey testing is supposed to be more rigorous, yet here we find an indication the opposite is true--an astounding 400-fold difference of papers and mentions in the *independent* peer-reviewed scientific literature (the only validated indications of safety).

This broad proof of the failure of FIFRA to ensure even reasonable safety is corroborated by an unkwown (to us) 1982 Congressional Report, which reportedly found that 60%-70% of a.i.s were not adequately tested for teratogenicity, 82%-85% were inadequately tested for carcinogenicity, and 90%-93% were inadequately tested for mutagenicity, and 100% were inadequately tested for immunotoxicity and hormonal effects; before the FIFRA'88 Ammendments which required every existing pesticide formulation to be re-registered, a task still largely uncompleted. The National Academy of Sciences also concluded in 1987 that only 10% of the total ingredients in pesticide formulations had been adequately tested for safety.

Aside from overwhelming the regulator’s capacity to evaluate pesticide risks, the other fundamental reason this friendly regulatory system has evolved is that the pesticide registrant, with their enormous economic interest in obtaining a registration, performs almost all safety tests. Registrant’s tests are performed for their own purposes, thus they are seldom submitted to independent, expert peer review--published in refereed (peer-reviewed) journals--as almost all valid science is; nor are they required to be.[466] In fact this data is often claimed as confidential (e.g. concerning other ingredients in formulations, see below)! There is also a significant record of fraudulent testing, documented immediately below. Moreover, an analysis found that two-thirds of the highest level officials in EPA's Office of Pollution, Prevention & Toxic Substances (OPPTS, which encompasses EPA's Office of Pesticide Programs), from 1977 to the present, are currently recieving some or all of their compensation from pesticide manufacturers (and 100% of 12 lower managers had

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[462] Committee on Scientific & Regulatory Issues Underlying Pesticide Use Patterns and Agricultural Innovation, National Research Council, Regulating Pesticides in Food; The Delaney Paradox (Washington, DC: National Academy Press, 1987).

[463] L. Burmeister March 1981 'Cancer Mortality in Iowa Farmers, 1971-78' JNCI:66:3;461-4.

[464] Cox December 2002, which says this is proved by the large percentage of recent registrations that EPA admits lack the required tests, others with tests waived, and many known to have dangerous effects (as described in this and other NCAP analysis of EPA data, summarized in the text above).

[465] EWG 1995. NLM’s Toxline indexes additional journals where additional safety studies of the two fungicides-only may be found, but our experience searching pesticide toxicicty literature using both interfaces indicates that the number of cites for them would not have reached 100 each.

[466] 40 CFR 152.94.

gone to work for pesticide manufacturers.[467]

Given the almost universal use in registrations of financially-interested, unpublishable data [a small fraction of registration studies are published (a great advantage to the party with a great finaioncial stake in their pesticide being declared safe), but we have never seen one in an independent journal (i.e. with adequate peer-review and conflict-of-interest standards). So it is logical to assume that significantly more fraud occurs in registration than has been documented. Over time, such llinks become visible. A review of the 115 published studies of bisPhenol-A's low-dose effects (bPA is, inter alia, an ingredient in some herbicide formulations) found while over 90% of the104 independent studies found toxicity, none of the 11 industry funded ones did!

Prosecutors and government investigators of the private Industrial Bio-Test lab (IBT) found that IBT had shredded paperwork *after* a federal grand jury was impaneled to investigate fraudulent chemical testing.[468] They were never able to determine how many of the tens of thousands of toxicity tests this company did (and pretended to do) for government agencies were valid and how many fraudulent. Criminal convictions against corporate officers were nevertheless obtained in 1983)[469] The IBT investigation/trial's cardinal revelation was to reveal how cozy the world of Q-RA testing is. That stems from the obvious and immense economic conflict-of-interest that both parties have. Monsanto, e.g., installed a manager of theirs at IBT and ordered test results for at least some of their chemicals to be faked,[470] though most of the fakery at IBT could not be traced to--or necessarily originated with--clients.

After the IBT scandal some labs who would not improve their practices refused to withdraw from the chronic toxicity tests market--until criminally indicted. Many others folded. Still, by 1983, "one EPA official concedes, there [were] still three or four testing centers that have a record of sloppy work or of losing data."[471] EPA found that IBT "performed" at least 800 pesticide registration tests of significant importance on at least 140 pesticides[472] (there are 600 a.i., each requiring various tests). While EPA later insisted that more than the initially identified 18% of 1205 key studies would eventually be found valid (through re-testing, if necessary), an EPA review of their IBT problem estimated that 95% of these 1205 tests were of "poor quality".[473] In fact, EPA's Office of Pesticide Programs (OPP) told the news media they did not use to audit the tests submitted by manufacturers in support of pesticide registrations.[474] The IBT fraud, affecting more agencies than EPA, led to the passage of the Good Laboratories Practice Act in 1978, requiring agencies to more stringently oversee labs doing tests for them.

Yet despite that increased lab oversight by EPA, a Craven Laboratories, its owner/president and three (later 14) employees were indicted in Austin Texas, Sept. 1992 on 20 felony counts of pesticide food residue testing fraud; resulting in convictions (e.g. $15 million and 5 years jail for the owner).[475] The IBT and Craven Lab test scandals together did caus some improvements in government oversight of pesticide and chemicals-in-general testing; but, as documented in the above critique of Q-RA, goverment auditors have found little improvement and that many gaps remain in this conflict and data-gap-ridden honor system of chemical Q-RA.

The capture by the pesticide manufacturer industry of their regulators (EPA) continues today. When their campaign contributions (agrichemical manufacturers gave $1.6 million in hard donations to federal candidates during the two-year 2002 election cycle[476]) or their revolving agency/industry employee entrance does not take care of their concern, closed-door meetings with EPA staff responsible for registration may be required, especially in recent years, when EPA has begun to review many old registrations. One such re-registration is for creosote (PAHs), registered for wood preservative uses. Previously we used the recently discovered toxicity of individual PAHs in these complex mixtures as an example of

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[467] Environmental Working Group ~1999 'From Bureaucrats to Fat Cats' Available at: http://www.ewg.org/reports/fatcats/fatcats.html (accessed 21 Jan. 2004).

[468] E. Marshall 1983 'The Murky World of Toxicity Testing: four scientists are on trial for fraud...' Science 220:1130-2; **also**: K. Schneider 1983 'Faking It: the case against Industrial Bio-Test Laboratories' Amicus Journal, Spring 1983, p.14 et seq.; finally, see Dawson 1982.

[469] E. Marshall 1983b 'Fed. Court Finds IBT Officials Guilty of Fraud' Science 222:488.

[470] Marshall 1983b.

[471] Marshall 1983b.

[472] U.S. EPA/Office of Pesticide Programs (OPP) 1983 'Summary of the IBT Review Program' Wash. DC.

[473] Marshall 1983b.

[474] Dawson 1982.

[475] U.S. EPA 1994 'Press Advisory: Craven Laboratories, owner and 14 employees sentenced for falsifying tests' Wash. DC.

[476] Rebecca Clarren 29 Dec. 2003 'Fields of Poison' The Nation, p.23.

Q-RA's failure to evaluate real-world exposure to chemical mixtures. EPA has been reregistering creosote during this time. Only the creosote industry was specifically consulted as EPA developed and risk estimates for their creosote exposure scenarios. The resulting draft allowable risks (i.e. 'safe dose') are one in 10,000 for cancer and 100 times the RfD for non-cancer effects (most Q-RA use one-in-a-million to define excess cancer risk!). Yet EPA's draft estimated risks of exposure to creosote as a wood preservative often exceed even these lax standards, e.g. one-in-a-thousand or more cancers for handlers of creosote--and there's no indication that EPA used the new knowledge of greater carcinogenicty of other PAH components.[477]

All that is what FIFRA still allows, despite decades of incremental improvement. Such results are inevitable when working under a law which 1) requires the balancing of complex risks with raw monetary values, and 2) relies on the party with the large conflict of economic interests to supply the data necessary to determine safety. This evolved system of pesticide regulation (permit the use of the poisons, use the biosphere as the real testing ground for their toxicity; then slowly ban the worst actors and slowly upgrade the statute's registration toxicity tests--often after the profits of patent protection have expired) has not changed. That is proved by the recent re-registration of the most widely used agricultural herbicide, atrazine. Despite robust new evidence of carcinogenic, reproductive and developmental effects, often at doses significantly below the levels it is ubiquitously found at in drinking water (concentrations that also far exceed the Safe Drinking Water Act's limit, affecting about a million people), EPA just allowed its continued use, with only minor new use restrictions.[478] As always, use is continued as EPA announces it continues to study such new evidence (in apparant violation of FIFRA's mandate to determine "no unreasonable risk" before allowing use).

In fact, almost every pesticide product cancellations has been voluntary (see our critique of Special Review, below at the end of the specific critique of the registration regulations): only when a manufacturer has an alternative poison ready for sale do cancellations seem to happen.[479] Keep in mind these broad reasons for this safety regulation failure when reading of the many loopholes in the registration rules themselves.

In short, it is clear that pesticide use and regulation are geared to the pesticide manufacturer's needs; not to the victim's (the biosphere, including the public) needs. FIFRA's core requirement of determining "no unreasonable risk" before allowing use is proven here to be fundamentally incompetent. Rather than spurring the fix of this rotten system, this well-known regulatory failure appears instead to have been covered up as far as possible, in an effort to keep the status quo of pesticide registration going.

Indeed, pesticide registration is no warrantee of safety. All pesticide sellers and users--such as the USFS here--would do well take notice of the competence and the attitude of the agency whose job it is to determine whether there is "no unreasonable risk".

One consequence of the popular misconception, that pesticide registration warrantees safety (combined with the extreme dangers these deliberate poisons tend to exhibit) has been the creation a very active tort court docket. As with many of the broad-scope regulatory federal statutes in our federation of states, the degree of warranty that FIFRA registration provides is constantly debated (including whether registration, and following the resulting label directions, preempts tort claims in state courts). *But in no circumstance does FIFRA allow claims of safety to be made, because it does not require that pesticides be safe, even when used as the label requires*.

However, because there is so much liability attached to the manufacture and use of these poisons, EPA does attempt make this known--that neither registration nor following the label's use directions preempt tort claims. For example, a formal Pesticide Registration Notice[480] is devoted to warning that EPA does not even consider non-target damage when registering pesticides; and it warns generally that registration is no warranty of safety.

In fact, tort claims of damage from pesticide use fit precisely into Congress' scheme for FIFRA. Over the years it has decreed in various reinforcing ways that both EPA and registrants must continually update the basis for the "no unreasonable risk" determination, as new information becomes available. It seems obvious that Congress meant for risks

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[477] Goldstein et al. 1998 **and** Nesnow et al. 1998.

[478] Cat Lazaroff 3 Feb. 2003 [untitled] Environmental News Service.

[479] E.g., this line of deadly chlorinated insecticides, sequentially banned over many decades: DDT, toxaphene, heptachlor, chlordane and chlorpyrifos. All these toxins persist in the biosphere, including in humans. A quick search of huge toxicology databases, eg Medline; even restricted to the last few years; will show you what a toxic problem these "banned" poisons remain in the biosphere.

[480] P.R. Notice #96-4 (1996), available direct from the home page URL of EPA's Office of Pesticide Programs.

identified via state-level torts be allowed, so that they may be included in pesticide registrations and bans (inumerable instances of pesticide damage have occured, some of which have been documented in court). The single instance of explicit testimony by a Representative or Senator on this question supports the use of state torts in Congress's FIFRA registration scheme. Actually, state torts are *crucial* in helping FIFRA erase the vast documented failure of the "no unreasonable risk" standard.[481] Common law in general also supports the use of state torts in the presence of a comprehensive federal regulatory scheme (FIFRA), including under theories of misbranding (given that 'The pesticide label is the law.').

The U.S. Supreme Court finally ruled (Bates v. Dow Agrosciences LLC; 2005) that FIFRA does not preempt state torts, in a pesticide damage case from Texas (in 2003 it declined to reconcile opposing judicial decisions as to property and personal injury claims; and the 5th Cir. Ct. of Appeals made a pro-federal preemption). EPA's brief on the question shifted with the party holding their top staff appointments--in 1994 EPA was telling courts that it believed that torts aid the goals of FIFRA (i.e. that that FIFRA sec .136(v)(b)categorically does not preempt damage claims in state courts; as a plain reading of that and the preceeding sentence seem to make clear, but last year the Solictor General told the Supreme Court that the federal agencies no longer take that position).[482] In any case, in a 9-0 & 7-2 decision, the Supreme Court ruled comprhensively and in strong terms that both harm and efficacy pesticide torts are allowed, because indeed FIFRA provides no warrenty of safety or efficacy.

Montana's Supreme Court recently allowed pesticide damage claims to proceed in state courts; not just for the non-target effects that EPA does not review, but also for ecologic or health claims.[483] Among other things, it was apparently not the intent of Congress in FIFRA to nationally preempt damage claims in state courts. All of the flaws and weaknesses of registration's basic "no unreasonable risk" standard that are documented throughout this section are essentially admissible as evidence to support tort claims in Montana and quite a few other states.

Relevant to the herbicides, especially those used by land managers in the West (e.g.USFS) is the recent consent decree between Dow Chemical and the NY Attorney General--including the largest enforcement fine in FIFRA history--to settle allegations that Dow blew-off an earlier 1994 consent decree with New York that was supposed to settle allegations that Dow made persistent false claims about the safety of its deadly (and commonly used in homes) insecticticide chlorpyrifos. Soon after that agreement Dow resumed illegal and expansive claims about pesticide safety, continuing until March 2003, even in the face of the NY AG's renewed investigation!

In this new settlement Dow, in addition to defiantly renewing illegal claims about the insecticide chlorpirifos, was also caught advertising illegal false claims about its herbicides; e.g. that Garlon 3A (triclopyr) is safer than caffeine and table salt (an old lie which ignores all health effects except poisoning deaths). Other illegal herbicide marketing phrases it used:
"herbicides are tested thoroughly..for their safety",
"they [pesticides] are environmental medicines",
"no risk when used as directed",
"not harmful to the environment".[484]

In the wake of the Clinton Executive Order to federal agencies to redouble invasive species control efforts, Dow--already the preeminent herbicide seller--targeted the West's noxious weed problem as a profit center, allegedly setting a target there of an extra $100 million of profit.[485]

Beyond the invalidity of *registration* as a warranty of safety it is also inane to claim--as this FEIS repeatedly does--that following pesticide *label* directions prevents or mitigates risks; or that it shields against legal liability for problems caused by pesticide use. FIFRA specifically prohibits the distribution or sale of pesticides that are misbranded (7 USC 136(j)(a)(1)(E)), including false or misleading representations of any type (7 USC 136 –definitions); and prohibits distribution or sale using claims that substantially differ from the claims made to get it approved (registered). The definition of misbranding also requires a "…warning or caution statement that is adequate to protect health and the

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[481] Bates Environmental Amicus Brief (for Beyond Pesticides, PSR and Sierra Club), accessed Dec. 2004 at: http://beyondpesticides.org/documents/BP_Bates_Amicus_Brief.pdf).

[482] All these recent decisions described in several stories in Pesticide & Toxic Chemical News:31:36 (30 June 2003).

[483] MT Sup. Court. cases # 99-185, 98-678 and 96-531

[484] New York Office of Attorney General 15 Dec. 2003 'Dow Subsidiary to Pay $2 Milion For Making False Safety Claims in Pesticide Ads' (Press Release, including appendix).

[485] Will Snodgrass (community activist), personal communication 1999, Missoula MT

environment".

It is true that it is the intent of the label's use directions to ensure that the "no unreasonable risk" determination is met. FIFRA 2(ee) sets the standard for labels:

"...use of...any registered pesticide in a manner not permitted by its labeling...".

Yet widespread use on labels of words of ambiguous enforceability was a key problem driving EPA to create its Label Review Manual, "..to improve the quality of labels and the consistency of their review."[486]

Additionally, *advisory* use practices on the label must enhance only the *mandated* practices and use restrictions on the label that were determined necessary to achieve the fundamental "no unreasonable risk" standard. Therefore mandatory label statements must be stated using imperative verbs ("shall", "must", "Do not", "keep away from"; etc.). The advisory label statements, on the other hand, must now be stated using clear, simple descriptive statements of the use practice (e.g. "latex gloves provide the best protection.").

Instead, labels commonly state advisories using words as: "should", "may" "recommend" (e.g. "latex gloves are recommended."). Such words are utterly ambiguous when it comes to determining what practices are mandatory to ensure that there is no reasonable risk, thus they have served as successful but improper (according to EPA) defenses in label enforcement actions.[487] Also, up until this brand-new Guidance, EPA had allowed a pesticide's manufacturer to change its label advisory statements at whim, and notify EPA after the change (now, EPA must approve the change/addition to advisory statements, in accordance with the Guidance).

Yet, these FEIS' at least once states categorically: "...adherence to label specifications for herbicide application would prevent exposure to toxic levels of herbicide."[488] Does "specifications" include the label advisory statements, or not? Moreover, there are generalized assurances throughout the FEIS that following the label will prevent or minimize risks, which never declare any intent whether or not the USFS will abide by the advisory statements.

The fact that detailed use directions--often several pages--are needed to ensure there is "no unreasonable risk" is a general indication that that standard is very hard to achieve in practice when allowing the use of very poisonous compounds.

What follows is a specific critique of the individual regulations (in their sequence) that implement the pesticide registration process.

• [40 CFR 152.91 and 158.45] The waiver of any data requirement may be granted on request from the economic beneficiary (the registrant). Waivers are massive in scope and routine; e.g. EPA's waiver from any of the critical chronic toxicity test for any pesticide that is not to be used agriculturally (thus it wont present dietary exposures), even though there may be no clear distinction between agricultural and non-food uses [see 40 CFR 158.240 to 158.740, the data requirement tables]. Billions of home applications of pesticides, for example, are made annually, exposing millions of vulnerable fetuses, infants and children. See also the discussion of 40 CFR 152.11 et seq. below (conditional data submission).

• [40 CFR 152.94] Data submitted need not be published in refereed journals that use independent peers to validate a paper's claims. Also, EPA will accept tests that use any "generally accepted scientific methodology" that EPA has not specifically determined to be invalid [40 CFR 152.83(e)]. These weaknesses might matter less had EPA the resources (and perhaps the desire) to oversee this crucial data's quality (the data to determine "no unreasonable risk"); basically: sufficient unannounced inspections, audits and credible sanctions. As documented above, the opposite is true.

• [40 CFR 152.91] If a data gap on an adverse effect exists in all literature, the effect need not be studied. The rule is intended for newly discovered issues or effects, but EPA need not, and frequently does not, set a time limit to submit data on the effect.

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[486] EPA Label Review Manual, abstract.

[487] EPA/OPP 2000 'Guidance for Mandatory & Advisory Labeling Statements, Pesticide Registration Notice 2000-5.

[488] FEIS p. 4-28.

• [40 CFR 152.111 et seq. to end of regulations] FIFRA sec. 3(c)(5) and (7)) allow either full (unconditional) or conditional amounts and types of data to make the registration determination. Conditional data registration means that EPA determines which, of the complete set of data requirements it lists, need be submitted for a given pesticide and its proposed use(s) to be registered for use. Even completely unstudied (new) chemicals may be registered using conditional data, though the registration is for a limited (yet unspecified) time [40 CFR 152.114 & 115]. Generally, food-use pesticides (an ill-defined, ambigous set) require more toxicity tests (such as critical developmental tests), on the theory that there will be additional and ubiquitous exposure through food.

Even the few, inadequate chronic toxicity tests required (see below--they are the best hope for detecting effects from widespread low-dose exposure) are only required for pesticides used directly on food crops [40 CFR 158.340--part of the data requirement tables]. Therefore some or many of all the non-food use pesticides may, at EPA's discretion, have been allowed for use without those essential tests.

Many, many other effects are only conditionally required to be tested. Again, EPA does attempt to determine the need for a test on the basis of a pesticide's uses and characteristics (e.g. its water solubility). But with so many potentially relevant tests, EPA may miss relevant ones. For example, of the few chronic toxicity tests that can be required, neither the reproductive or teratogenic (birth defect) tests are required for non-food use pesticides, if the economic beneficiary (the registrant) does not anticipate exposure to women of child-bearing ages [40 CFR 158.340] and EPA agrees. Similarly, no carcinogenicity test is required for non-food use pesticides if the economic beneficiary (the registrant) submits that any human's lifetime exposure is unlikely to be significant [40 CFR 158.340].

• [40 CFR 152.116] Data need not be submitted if employing FIFRA's 'exclusive use' exemption. This apparently can exempt from tests any a.i. used in a single way only.

• [40 CFR 158.102(b)] Explicitly states that the composition of the a.i. that is sold may be assumed to be the composition of the a.i. that is tested by the economic beneficiary (the registrant) for registration. Sometimes EPA even allows a purified a.i. to be tested [scan the 'test substance' column of the data requirement tables, 40 CFR 158.240 to 158.740]. Most often, e.g. for all of the critical human and most of the ecological toxicity tests, EPA allows the "technical grade" of the a.i. to be tested--supposedly what is produced for manufacture, but in fact the technical grade tested is created before full scale production begins (except in the case of re-registration or Special Review), and small batches tend to have less contaminants. Even if the technical grade comes from the production line care might well be taken, knowing it will be used for toxicity testing. The 'test substance' column of the data requirement tables shows that in only a few use or product circumstances does EPA require testing the product that is actually manufactured, formulated and sold.

EPA does claim the discretion, though not the obligation, to verify the composition of a substance tested (whether it be a purified a.i. or an 'as-sold' product), which composition the economic beneficiary (the registrant) must certify [40 CFR 158.150(b)(2)]. However, the requirements to test for impurities [40 CFR 158.155(c) to (f), and 158.167 to 158.180] are extraordinarily lax. For the a.i. only, the economic beneficiary (the registrant) initially determines which ones are "toxicologically significant". Otherwise the registrant does not have to worry about impurities so long as the impurity is below 1,000 ppm by weight of the a.i., a very high level. E.g., the potent carcinogen hexachlorobenzene impurity in the herbicide picloram, actually allowed to reach just 200 ppm, still exceeds the acceptable cancer risk for some applicators.[489] Last, impurities of "inert" ingredients in formulations are almost completely ignored [40 CFR 158.155(f)].

In short, the failure to effectively control impurities during registration is a critical issue because they may have unstudied risks or may be known to be extremely toxic (such as the extremely potent carcinogens hexachlorobenzene, nitrosamines and dioxins in the herbicides at issue in this FEIS (we describe their risks elsewhere)). EPA in 1998 let registrants self-certify the validity and sufficiency of their pesticide formulation composition tests.[490]

• 40 CFR 158.155(b) **"(1)...Generally, it is not required that an applicant know the identity of each ingredient in a mixture that he uses in his product."** It is an irony befitting the entire burlesque of FIFRA registration that EPA dubs "inert". all but one (at times, two or more) of the intentional ingredients in a formulation.

Therefore pesticide risk evaluation only applies to ingredients the registrant deems to be 'pesticidal', called the active ingredient or a.i., with a few minor exceptions. Of the 2,300 inerts disclosed or known to EPA, EPA still classes the

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[489] EPA Picloram Re-registration Eligibility Document (RED)'s EPA factsheet.

[490] Pesticide Registration Notice #98-1.

majority, 1,700, as having unknown risks![491] Common “inerts” include highly dangerous solvents (e.g. xylenes and ethyl benzene) and detergents (e.g. the low-dose hormone-disrupting alkyl phenols). Their quantity, their toxicity; their synergistic effects; their effects on the formulation’s environmental partitioning, metabolism and other environmental behavior parameters; can be ignored simply by falsely labeling them ‘inert’. In fact at least 26% of the inerts EPA knows of (about 600) have been classified by governments as hazardous,[492] aside from their toxicity data gaps. Of 1,800 inerts, 75 are considered hazardous by the CAA, 52 by Superfund, 43 by EPCRA (the TRI), and 78 by TSCA.[493]

Despite the entire purpose of FIFRA being to control the risks of a.i., EPA allows banned a.i.’s to be used as these secret “inert” ingredients. For that matter any a.i., such as one that still has only an ancient (all but useless) registration may be used as an inert. Comparing EPA’s lists of known inerts to EPA’s lists of a.i. NCAP discovered 394 a.i. listed as inerts--16% of all listed inerts![494] Eleven are restricted-use pesticides, and only two of the 394 are currently categorized as toxic enough “inert” chemicals that EPA requires their listing on the label with the alongside the a.i. They’ve since updated that to almost a quarter of al listed inerts were at one time or currently used as an a.i.[495] Of inerts with unstudied toxicity, 292 are registered as a.i.[496] In short, these “active inerts”, as NCAP has dubbed them, are an easy way for pesticide makers to dispose of stocks of imported, banned or voluntarily canceled pesticides, such as DDT.

• [40 CFR 158.202(e)(1)-(3)] This rule correctly explains that chronic toxicity tests are needed to evaluate repeated doses for a prolonged period. As explained in the preceding major section on quantitative risk assessment (Q-RA), chronic effect toxicity tests obviously should strive to represent the far more typical low-dose exposures. Also obviously, EPA should test real-life mixed chemical exposure, and evaluate all health end-points. (Regarding the rarer acute (i.e. high-dose) risks, the EPA backed down in 1996 from a proposal to let the economic beneficiary (the registrant) self-certify that these tests were valid and sufficient--i.e. the EPA would have not have even reviewed this data).[497]

• [40 CFR 158.34 and 158.340] Chronic toxicity.[498] The reason that this FEIS only evaluates directly a paltry number of chronic effects (four--carcinogenicity, teratogenicity (birth defects), reproductive effects and mutagenicity[499]) is that those are four of the five chronic toxicity tests that pesticide registration requires (conditionally). The fifth is the general chronic toxicity test, which looks for effects in many of the major organs, and since the results of this test cannot be summarized in a word or two, we assume that is why this FEIS did not list it. Our criticsms in "Problem #4" of our critique of chemical Q-RA above aply to FIFRA's general chronic toxicity test , as pesticide registration tests use the same protocols as other chemicals.[500] Together, these five chronic toxicity tests required (usually) to register a pesticide a.i. fail to look at the thousands of distinct biological functions that could be affected, in order to meet the fndamental FIFRA mandate of determining that a pesticide a.i. poses no unreasonable risk.

So, first, these FIFRA tests fail to evaluate inhalation or dermal exposure routes, which account for much of pesticide exposure.[501] Most important, they only generate information from visual ir microscopic (sometimes) viewing of some of the tissues of many--but not all--the major organs. Thousands of biochemical functions are ignored. Major systems such

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[491] Known inerts are categorized by known (or unknown) risks at http://www.epa.gov/OPPrd001/inerts.xls

[492] Marquardt, Cox & Knight 1998 ‘Toxic Secrets: ‘inert’ ingredients in pesticides, 1987-1997’. Northwest Coalition for Alternatives to Pesticides, Eugene Oregon (home pg., http://www.pesticide.org).

[493] R. Renner 15 Oct. 2005 ‘(Perspective) Are industry’s secret “inerts” an unrecognized environmental danger?’ Env’l Sci & Technol:39: 417A–8A.

[494] NCAP Jan. ‘98 ‘Worst Kept secrets: toxic inert ingredients in pesticides’, p. 3.

[495] Carline Cox Spring 2005 ‘When Inert Is Not Inert’ J Pesticide Reform:25:2:2.

[496] R. Renner 2005.

[497] Ntl. Coalition for Alternatives to Pesticides (NCAMP) Feb. 1998 ‘EPA Reduces Oversight of Pesticide Producers...’ Technical Report 13:2:3.

[498] The detailed standards for conducting any required test are actually in separate ‘Pesticide Assessment Guideline’ documents, which the CFR cites--see 40 CFR 158.108. EPA also has formal Guidance for health effects testing that applies to all U.S.A. and OECD-country health effect tests. See EPA/OPPTS 1998.

[499] Mutagenicity is tested more as an acute effect, but a positive result is a flag for chronic effects, because mutagenicity means damage to DNA, which obviously can lead to any disease. Ditto for clastogens, agents that damge the chromosones that DNA arranges itself into. It is critical to keep in mind that toxic agents can modulate DNA to cause mistakes, while not damaging it. This is how many hormones (and hormone disruptors) act, in part.

[500] EPA/OPPTS 1998 (plus EPA’s other chronic effect test Guidances).

[501] EPA/Office Research & Development 1990 ‘Project Summary: Non-Occupational Pesticide Exposure Study (NOPES)’ Research triangle Park, NC.

as hormones, immune and male reproduction are not tested, even in this primitive way. There is also no test of chronic neurologic damage, except for occassinal (case-by-case) decisions by EPA to test an a.i. for neurologic damage during development (a critical period).

Reproductive and teratogenic tests (two of the five categories of chronic toxicity testing in FIFRA) dose a pregnant female and evaluate effects on the fetus after its birth, or to see if it is spontaneously resorbed (therefore, an additional failure of the reproductive test is that it actually does not test for male reproductive effects--even the originally dosed generation). In both these tests, chemical damage to the fetus' genes or to its reproductive system may manifest only in the third generation--the offspring of the fetus--or in the fetus' failure to be sufficiently fertile. In fact, genetic damage to the fetus could cause almost any effect in the 3rd generation, not just birth defects. These registration requirements [at 40 CFR 158.202(c)(3) and 158.340] do not require 3rd-generation and call for two-generation tests, and EPA infrequently requires 3rd-generation testing.[502] The many estrogenic or other sex hormone-disrupting pesticides and other chemicals are logical candidates for causing such third-generation effects.[503]

As proved in our critique of chemical Q-RA, these and all other chronic toxicity tests have the following failures. Perhaps the largest failure is their absolute assumption that no effects can ever occur at the real-world doses we ubiquitously receive. The far more vulnerable period of development is barely tested. Toxic synergy between the stew of chemicals in the biosphere and inside of organisms is not tested--even when other, untested, chemicals are added to an exposure, as is the case with pesticide formulations. Inter-species and inter-individual variations in response to toxics are not dealt with effectively. There is no effective monitoring of exposures, and of just a few diseases. Last, testing fraud is endemic in these chronic toxicity tests, driven by the large economic interests of the chemical sellers.

This failure to fairly evaluate chronic injury--not just in humans--is the core of the failure of pesticide registration to guarantee "no unreasonable risk". Historically, it takes registered pesticides to show an effect for EPA to begin to test for the effect. Tests for cancer, mutagenicity and reproduction were not required until the 1960s &1970s after those effects become known in the 1940s and 1950s.[504] In keeping with this tradition, EPA currently is taking over a decade to implement tests immune and hormone injury, while it still does not address the other gaps in potential end-points affected.

• [40 CFR 158.490] Wildlife and aquatic organisms. Non-human species receive even less consideration from pesticide registration. Only a single terrestrial and aquatic vertebrate species receives a chronic test (the more realistic, lower-dose exposure scenario), looking for gross effects in organs and for just a handful of specific diseases. The remaining parts of an ecosystem are hardly tested at all; if they are, it is almost always with the ridiculously un-informative acute lethal dose test.Wildlife have far, far less energy at their disposal than do humans to acquire an adequate diet and habitat, yet FIFRA requires no evaluation of a pesticides effects on food resources or habitat. Critical behaviors such as predator avoidance, homing and parenting are not evaluated for interactions with pesticides. For example, adding a predator to water containing tadpoles and low (typically encountered) levels of the common insecticide carabryl (Sevin) strongly synergizes the acute and mid-term (10-15 day--not tested under FIFRA) lethality of the carbaryl on the tadpoles of several frog species.[505]

Moreover there is no requirement to test for the effects of a.i. on soils and their microrganisms, the critical base of all ecoystems. As always, as independent scientists begin to explore this data gap out of curiosity, they are finding severe risks indeed. E.g., it turns out that the signal phytoestrogen/receptor complex that is critical for plant roots to fix nitrogen from soil via sa ymbiotic relation with bacteria (a fundamental necessity of many ecosystems) is disrupted by 45 of 62 known hormone disrupting synthetic toxic chemicals (most were a.i. or pesticide formulation ingredients), by inhibiting the production of this protein/receptor complex.[506] A broader lesson from this shockingly widespread result is that chemicals just begining to be studied for their effect on vertebrate's estrogen receptor (with unnumerable roles) should also be studied for their effect on the much larger non-steroid receptor, non-mammalian world![507]

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[502] Caroline Cox 20 Dec. 2002 personal communication with Tony Tweedale, Missoula MT.

[503] One famous example--the result of a similarly flawed FDA test protocol--is the drug diethyl stilbestrol (DES, a potent estrogen), which causes devastating reproductive effects in 3rd-generation humans.

[504] Cox 2002

[505] R. Relyea 2003 'Predator Cues and Pesticides: a double dose of danger for amphibians' Ecological Applications:13:6:1515-21 **and** R. Relyea & N. Mills 27 Feb. 2001 'Predator Induced Stress Makes the Pesticide Carbaryl More Deadly to Grey Treefrog Tadpoles (Hyla versicolor)' Proceedings Ntl. Academy Sciences:98:5:2491-6.

[506] Jennifer Fox et al. May 2004 'Phytoestrogen Signaling and Symbiotic Gene Activiation Are Disrupted by Endocrine Disrupting Chemicals' Env. Health Perspectives:112:6:672-7.

[507] Jennifer Fox May 2004 (Commentary) 'Chemical Communication Threatened by Endocrine-Dirsrupting Chemicals' Env. Health Perspectives:112:6:648-53.

In fact, a 1995 review (with 262 references) of the impacts of *herbicides* alone on terrestial flora and fauna, found massive data gaps throughout the world, yet especially in N. America. It did find sufficient literature to prove that the use of herbicides has dramatically altered the habitat pattern of temperate landscapes in North America and western Europe, with strong evidence for adverse effects of changes in habitat pattern on beneficial insects and arthropods in the United Kingdom, and on birds in North America and western Europe.[508]

• [40 CFR 158.540] Damage to other simple species--non-target plants--also receive little consideration in pesticide registration. Few pesticides are tested on plants beyond a first, utterly rudimentary tier of effects. Among the effects ignored is plant reproduction, which many pesticides affect (such as the potent sulfonureas proposed for use here). Pesticides--especially herbicides--both increase and decrease the production of phytoestrogens and the other important compounds that plants (and some other low level organisms) use to send internal and external signals; a troubling but vague result that indicates how unstudied these portions of ecosytems are.[509]

• [40 CFR 158.590] Next in this succession of care-less-ness, non-target insects are accorded the least test consideration of all. It may be that EPA is too busy approving pesticides to finish the insect test protocols it says, in this section of the rules, that it is developing. As always, the more realistic chronic effect tests (for insects, pending) are minimal. Finally, the rest of an ecosystem, including the base (microorganisms and fungi) that the entire ecosystem relies on, is not tested at all in determining whether a pesticide is safe enough to be sold.

In addition to these gaping loophles in the basic pesticide registration test requirements for non-mammal species, the Endangered Species Act (ESA) requires that EPA consult with U.S. F&WS or NOAA Fisheries (the agencies that manage listed (at risk) species), advising them of the risks that pesticide a.i.s pose to these threatened and endangered species. As always, EPA here has a backlog of more than 50 a.i. (out of ~700 total registered a.i.s) that it has not begun to perform even this minimal ESA review on.[510] Moreover, EPA is now proposing to gut this review, limiting it to a.i.s that it deems "likely" to pose risks to these at risk species (not even those a.i. it thinks are 'possible' threats to them) (this is especially galling when you consider the above proofs of how almost all species get no test during registration than the amount of the a.i. that immediatelly kills). Finally, according to this report and another,[511] the American Bird Conservancy has FOIA'd emails & memos that show a pattern of industry interference in this ESA review mandate of EPA's. E.g., manufacturers told EPA to delete any reference to eagles when reviewing rat poisons known to kill ESA bald eagles, among countless other species. Adding up all this obvious evidence, it is crystal clear that it is the manufacturer's and EPA's intent to hijack the basic FIFRA 'no unreasonable risk' mandate so that almost no data is generated that could add to the risk side of the balance, in order that the manufacturer's economic benefit may proceed (as they do in so many areas of FIFRA).

• [40 CFR 159] Sec. 6(a)(2) FIFRA requires registrants to report to EPA “additional factual information regarding unreasonable adverse effects” once a pesticide is registered. EPA promulgated a rule and published Guidance (Pesticide Registration Notice 98-3) clarifying and specifying what sort of information it believes is required under sec. 6(a)(2). Shortly after however, EPA issued a clarifications and corrections[512] that both opens new loopholes and makes explicit the weakness of this statutory requirement. In short, 1) information about future chronic effects need not be submitted (apparently throwing in doubt whether any study of chronic effects of a pesticide need be submitted); and 2) making clear that no-one but the registrant falls under this reporting obligation (not employees or agents of the registrant). This is definitional narrowing was done in the face of a federal court decision that EPA, not the registrant gets to decide what is an "unreasonable adverse effect".[513] Once again, the private party with the large economic interests is largely in control of the basic “no unreasonable risk” registration determination.

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[508] K. Freemark, Boutin C. 1995 Feb 'Impacts of agricultural herbicide use on terrestrial wildlife in temperate landscapes - a review with special reference to north america [review]'. Agriculture Ecosystems & Environment:52:67-91.

[509] O. Daniel et al. Feb. 2001 'Selected Phenolic Compounds in Cultivated Plants: ecologic functions, health implications and modulation by pesticides' Env. Health Perspectives:107(Suppl. 1):109-14.

[510] Jessica Snyder Sachs Dec./Jan. 2004 'Poisoning the Imperiled' National Wildlife, p. 22-29.

[511] Lisa Stiffler 8 Dec. 2003 'Critics Say Pesticide Makers Illegally Influence EPA Policy' Seattle Post-Intelligencer http://seattlepi.nwsource.com/local/151518_pesticides08.html , describing activists' intent to sue EPA for violating the Fed. Advisory Committee Act's restricitions on the influence of committees that advide federal agencies.

[512] EPA/OPP 1998 PR Notice 98-4.

[513] CSMA & NACA v. EPA, 484 F. Supp. 513 (1980, in the District Court of D.C.)

• In addition to *regulatory* exemptions from registration testing requirements,[514] there exist broad *statutory* exemptions, including “experimental” “local need” and “emergency use” exemptions, that in fact often continue for years and decades.[515] An additional “Minor Use” (under 300,000 acres) statutory exemption was expanded in Congress’ 1996 amendments to FIFRA.

• Finally, no critique of pesticide registration flaws would be complete without mention of FIFRA’s disastrous Special Review requirements for de-registering a pesticide when data becomes available that it may pose an unreasonable risk after all. The average Special Review takes five years (2,4-D’s, another of the popular herbicides in this FEIS, has been going on much longer than that, with the end possibly several years off) and EPA has only ever completed 45 special reviews.[516] This lengthy process, including the typical risk balancing tests of FIFRA, usually culminates in “voluntary cancellations” of a few or some of a pesticide’s registered uses; seldom all. These are negotiated agreements that typically allow for the sale of remaining stocks in the canceled uses, over long periods.[517]

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[514] See the registration data requirement tables, 40 CFR 158.240 to 158.740, which specify the testing data that must be submitted to EPA, for every use the pesticide is being registered for.

[515] EPA/OPP 2001 FIFRA Sec. 18 Emergency Exemptions (e.g.): http://cfpub1.epa.gov/oppref/section18/search.cfm

[516] EPA/OPPTS 1998 ‘Status of Pesticides in Re-registration and Special Review’ Washington DC.

[517] GAO 1992 ‘Pesticides: EPA lacks assurance that all adverse effects data have been reviewed’, T-RCED-92-16.

PICLORAM: a CASE STUDY of FAILURE of FIFRA's "NO UNREASONABLE RISK" MANDATE

Aside from this FEIS' many claims of herbicide safety due to registration and of the unquantified ability of mitigation to take care of any consequences that do occur, this FEIS claims that the impacts of herbicides on non-target vegetation will be transient[518] and outweighed by the benefit to native vegetation from herbicide use[519] (which ignores other methods of control and ignores that herbicide use is not an effective control in the long run, as proved above). The authorities to prove these claims are not given.

Dr. Peter Rice at the U. of Montana, Missoula MT, has accepted at least $24,000 dollars from Dow,[520] picloram's manufacturer, to do research on picloram's non-target vegetation toxicity. Picloram is the most widely used herbicide on western public lands, including those of the USFS.[521] It was recommended to be banned by EPA's experts on the environmental behavior of pesticides, due precisely to its persistence and toxicity in water and its non-target plant toxicity.[522] So, if glyphosate is even less selective than picloram, as this section of the FEIS says, what's the impact of glyphosate on non-target environments? Now we show just how non-selective picloram is.

The chlorinated pyridine family of herbicides are to be heavily relied on in this FEIS (picloram, triclopyr and clopyralid). The FEIS calls, without citation, clopyralid the most selective herbicide, i.e. the least harmful to non-target vegetation) are persistent (especially in water) and mobile in the environment; and kill non-target plants of intrinsic and economic value.[523] There are many instances of them contaminating groundwater and killing native plants and crops. Examples are that the state of Washington recently banned clorpyralid after several incidents of it poisoning plants and crops even after surviving the microbial and heat decomposition of compost; after which Dow was forced to announce the voluntary withdrawal of clopyralid's registration for use on residential lawns nation-wide.[524] Picloram was recently detected in the *drinking* water well of a St Charles, IL school, at over twice the allowable level.[525] Locally, a recent large native vegetation kill occurred at the BNF's 'Willoughby 40' Recreation Area after a picloram + 2,4-D application.[526] When considering whether to ban picloram (during its re-registration), EPA calculated that it exceeds the EPA 'substantial hazard' level to non-target plants by up to 13,000(!) times, depending on the application method.[527] the Chief of the Ecological Effects Branch of pesticide registration recommended against the reregistration (i.e., a ban) of picloram:

"This conclusion is based on the extreme exceedance of the acute levels of concern for non-endangered and endangered terrestrial plants."[528]

Critically, a recent local study found that a common picloram formulation, applied according to the label, kills native plant germination even two years after its application (using the soil half-life of picloram to derive the 2nd year's concentration, as there was an external time limit to the experiment),[529] confirming what the picloram label (and much of the literature the Forest cites, such as the Rice paper) agree is a major risk of picloram (non-target plant risk).

Thus, the consequences of herbicides on native plants have not been independently demonstrated in this FEIS. Because clopyralid and triclopyr are in the same chemical family as picloram and have similar persistence and mobility in the environment;[530] and given the multiple examples of clopyralid & triclopyr's effects on non-target vegetation, even after being on other plants and composted; we are highly skeptical of the FEIS' un-cited claim that clopyralid is the most

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518 FEIS p. 4-5.

519 FEIS p. 4-5.

520 U. Montana/Integrated Plant Management Committee 1998 'Mt. Sentinel Vegetation Management Final Plan' Sec. 4.17 (pg. 17). Will Snodgrass (Chemical Injury Information Network, Missoula MT) exposed this conflict of interests.

521 Cooperative Extension Services of MT, UT & WY '1999-2000 Weed Management Handbook'.

522 EPA/OPP 1995 'Re-Registration Eligibility Document (RED) for picloram' Wash. DC.

523 EPA/OPP 1996 'Picloram RED Facts', Wash. DC (the Re-registration Eligibility Document (RED) factsheet).

524 J. Pesticide Reform, Fall 2002, p.5. **Also** David Bezdicek, 6/04/2001 'New Herbicides Found in WSU Compost' Washington State U Compost Facility home page, index.htm --many other examples exist.

525 NCAMP/Beyond Pesticides Technical Rpt., May 2002.

526 Ravalli Republic, 6 June 2001, p. 1.

527 EPA/OPP 1995 (Picloram RED).

528 EPA/OPP, Special Review and Registration Division, Ecological Effects Branch undated 'Review of Picloram' (prepared for the 1995 re-registation of picloram).

529 Amanda Tripp 2003 'The Use of Blanketflower (G. aristata) As a Bioassay for Residual Tordon 22K', presented of MT Academy of Sciences Annual Meeting, Junior Section, 26 Apr. 2003, U. Montana Missoula MT (Big Sky HS student).

530 EPA/OPP 1998 'Triclopyr RED Factsheet';.EPA 16 May 1997 'Clopyralid; Pesticide Tolerance...' Fed. Reg. 62:26,949-26,954.

selective herbicide currently planned for use. We expect all of picloram's ecological dangers that led EPA scientists to recommend it be banned would be somewhat similar for the other members of this chemical family.

The other driver of the EPA scientists' desire to ban picloram is its persistence and mobility:
"...because its use would pose unreasonable adverse effects to the environment. Because of picloram's mobility in soil types and its persistence under normal ambient conditions, no practical use restriction can prevent it from contaminating the environment surrounding the target site...The use pattern of picloram is highly specialized, but it is almost certain to eventually reach ground water in areas where it persists in the overlying soil. In submitted terrestrial field and forestry studies, picloram exhibited calculated half-lives of up to 278 days and was detected up to the limits of sampling depth (up to 1.8 m)...Picloram has a high degree of phytotoxicity. ... [Picloram is] resistant to biotic and abiotic (e.g., u.v.) degradation processes. ...In some soils, it is nearly recalcitrant to all degradation processes."[531]

Thus picloram contaminates water easily--e.g. it was in 420 of 744 surface water samples in EPA's STORET drinking water database, found at up to 30 ppb.[532] A field study found that 6% of applied picloram left an 8-acre treated site over a month with several rainstorms. Measured at the entry point into the stream, it diluted to 5 ppb a full 5.4 km downstream.[533] Given that eight times that level (40 ppb) kills and decimates trout fry,[534] what were the aquatic effects of this washed-off picloram in the several km. upstream? A study of picloram's persistance and mobility, in Western Montana, found that picloram applied at recomended rates persisted and migrated downwards towards groundwater as far and as long as was measured (over 10% of application detected at one meter after 90 days (and increasing at this depth aas measurement ceased); 10% detected in top 12.5 cm of soil after 445 days at a second site),[535] a study that EPA scientists cited in their recommendation that picloram be banned. Finally, two week-appart applications of picloram a1/4 mile upstream of a fish hatchery killed 15,000 pounds of trout after a rainstorm flushed it into the water.[536]

The picloram RED Fact sheet's claim that picloram is almost non-toxic to birds, mammals and honeybees is based on acute lethality data only, which is irrelevant to most of these organisms (and to many other species), which on a regular basis receive pesticide doses that do not kill immediately. What does happen to these organisms? As explained above, the tables which specify what studies are needed to register a pesticide assume, for non-mammals, that anything short of death has to be safe--it's not tested for.

EPA's RfD for picloram is 0.07 mg/kg of body weight per day. But this Q-RA that the USFS is relying on uses a picloram RfD almost 3 times higher (less safe), 0.2 mg/kg b.w./day (which happens to be the same as a the manufacturer's, Dow's, calculated RfD, which EPA accepted in setting the level of picloram allowed in food (food tolerances). It's definitely not the regular, EPA-approved picloram RfD found in EPA's Integrated Risk Information System (IRIS) database. Even so there are a few modeled exposures in this Q-RA (which models exposures of pesticide applicators, the general human population, aquatic and terrestrial organism) that exceed the more lenient RfD. How many more would exceed it if they had used to stricter RfD?

Last, picloram always has hexachlorobenzene (HCB) contamination, a potent carcinogen and with a very low RfD for other health effects, as already described. As usual in Q-RA's, this risk is not estimated. It has been shown that wildlife (just as EPA calculated for human picloram applicators) are at great risk of cancer or other disease from HCB. Predators of ungulates eating only picloram-sprayed forage were, in a local Environmental Assessment (EA), calculated to ingest 0.021 to 0.034 mg/kg per daily meal of HCB--up to 43 times the HCB non-cancer 'safe dose' (RfD) of 0.0008 mg/kg/d. The ungulates were claimed to ingest less than the HCB safe dose, without any quantification; and the Finding of No Significant Impact (FONSI) to avoid an EIS was finalized![537] HCB in picloram alone accounts for 70% of EPA's allowable risk for HCB exposure.[538] Exposure to HCB of ground applicators of picloram exceeds EPA's acceptable cancer risk level by ten-fold (HCB causes cancer in test animals at very low doses: 0.02 ppb in drinking water is calculated to cause cancer in one-in-a million animals). These applicator's intermediate-term HCB exposure also

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[531] EPA/OPP, Special Review and Registration Division, Env. Fate & GroundWater Branch (EF&GWB) undated 'Review of Picloram' (prepared for the 1995 re-registation of picloram).

[532] EPA/OPP 1996 (Picloram R.E.D. Factsheet).

[533] Mayeux et al. 1984 'Dissipation of Picloram in Storm Runoff' J Environ Quality:13:44-8.

[534] D. Woodward 1976 'Toxicity of the Herbicides Dinoseb & Picloram...' J. Fish. Res. Board Can. 33:1671-16.

[535] Vicky Watson et al. 1989 'Env. Fate of Picloram Used for Roadside Control' J. of Env. Quality 18:198-205.

[536] B. Keyes 1992 'Going Fishin' With Tordon 22K' J Pesticide Reform:12:2:18-19.

[537] U. of Montana/Integrated Plant Management Committee May 1998 'Mt. Sentinel Vegetation Management Final Plan'. See response to comment #1.8 on p. 5.

[538] EPA/OPP 1996 Picloram RED.

exceeds the ATSDR's non-cancer safe dose level.[539]

HCB, allowed at up to 200 ppm in picloram (the most popular herbicide on public lands); persistent and already associated with several human reproductive disorders; was shown to significantly speed sexual maturity of the prostate in male mice at a low dose, environmentally relevant dose; yet it significantly retarded this development at a dose just two to greater than 20 times higher. In vitro, it was confirmed that the low dose HCB stimulated action via the androgen hormone receptor while high dose repressed it.[540]

Picloram's new label (re-registration was granted in 1995) contains many examples of ambiguous use directions, of the type that recently caused EPA to begin to crack-down on such un-enforceable label directions (see the above criticsm). "Application *should* be avoided [at winds] below 2 mph [to minimize direction shifts and temperature inversions]...and above 10 mph." This label advisory, in place of a clear use direction, means there is no need to worry about how to measure wind speed, or if it is possible to keep wind speed and direction constant. Picloram's label also, inter alia, "advises" not to apply to residential or commercial lawns; "advises" drift be avoided; and says to apply at rates "suggested" in the approved uses section of the label (though, still more ambiguously, application rates are elsewhere in the label mandated). These unenforceable label use advisories were issued by EPA in the face the original recommendation of its scientists to ban picloram, but for exactly the same reasons.

Instead of a ban, EPA over-rode its experts on environmental fate and ecological effects, and re-registered picloram. EPA restricted its manner of application somewhat (both with mandatory and ambiguous advisory label language), and called for several studies on these questions of picloram's risks.[541] As explained above in the general critique of FIFRA , calling for studies while registering a pesticide for use appears to be an example of an illegal registration: if more studies on these critical effects of picloram use are required, how on earth can EPA have determined that there is "no unreasonable risk"? The only other major factor in this registration determination is that of Dow's economic benefit.

In sum, picloram's persistence and mobility allow it to encounter all the life forms of an ecosystem, and it has been shown to be toxic to many of these organisms at the levels it is applied. As detailed elsewhere, weeds are developing resistance to it. Is it unreasonable to ban it, as EPA scientists wanted to?

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[539] EPA/OPP 1996 Picloram RED.

[540] Jody Ralph et al April 2003.

[541] EPA/OPP 1996 (Picloram RED Factsheet).

<u>'Risk Assessment for Herbicide Use in Forest Service Regions 1, 2, 3, 4 & 10 & on BPA Sites' Sept. 1992</u>

Of the numerous Q-RA that plaintiffs have read, this one is the first whose authorship, individual or corporation, is hidden, though the USFS' contract number is on the cover. It is possible that authorship was on a page that was not copied for the copy the plaintiffs received from the USFS (though if so, the possibility exists that many staff of the USFS & BPA do not know who is responsible for its claims). This Q-RA becomes even more opaque by failing to show any of its data inputs or outputs (other than the safe doses, used to compare estimated exposures to); nor does it say were they can be found (see p. III-E-2). Rather, qualitative tables and text describe alleged quantitative risk estimates. The heart of any Q-RA is its data inputs and the resulting outputs. Therefore this Q-RA not only refuses to show most of its inputs and all its results, it refuses to say who has them! (again, assuming they exist).

However, what is presented is enough to severely criticize the validity of its conclusions, because it does state its methodology, and it does give each chemical's calculated safe dose (with the source of it). We will assume that this Q-RA actually ran its inputs in a computer program that generated the risk estimates for the various estimated exposure routes, such that the missing quantitative data confirms the presented *narrative* summary risk tables. If that is the case, this Q-RA has the following fundamental flaws. Recall, as you read, that pesticides being evaluated in any particular Q-RA are unsafe from the start, due to the degree of problems described in the first two sections (chronic toxicity test and pesticide registration failures).

A) A classic Q-RA trick, employed here, is to substitute a metric called the "MoS" (margin of safety) for the non-cancer RfD 'safe dose' method. The MoS takes the RfD's built-in safety factors and explicitly calls them "safety margins", which are then highly emphasized and headlined in the text and in tables. The problem, described above, is that the safety factors of the RfD method are 100% arbitrary--they are not applied with reliable knowledge individual and species differences in response; and they certainly cannot compensate for missing toxicology data, which usually gets a weak 3 or 4-times the NOEL safety factor. For such reasons, the RfD metric does not advertise or emphasize that its calculated safe doses are in fact safe.

As an example of misuse of the MoS metric, assume that one of this Q-RA's estimated exposure from the herbicide uses it evaluates happens to equal the herbicide's RfD, set with the usual safety factor of 100 times less than the NOEL. The MoS metric says instead that the Q-RA's estimated exposure has a "margin of safety" (MoS) of 100 times the NOEL. This gives the inescapable impression that the Q-RA is saying with certainty that the exposure is very safe, whereas the RfD metric is designed to leave the impression that the same exposure level is right at the margin of the estimated safe/unsafe exposure level. Moreover, the exposure scenario evaluated in this Q-RA may be of an individual or a species (or both) that is more sensitive than the 100-fold "safety margin" granted.

Thus, the MoS trick causes all of this Q-RA's non-cancer risk estimates to appear about (depending on a herbicide's RfD's safety factors) 100-fold too low--not by changing the RfD, but rather by re-packaging it. Also, most Q-RAs that use the misleading MoS metric will at least show the RfD alongside the MoS, even as they emphasize the MoS number; but this is one of those that doesn't (in fact as mentioned, it doesn't even bother to quantify the MoS, most unusual!). In sum, not showing the RfD, while highlighting the presentation of the MoS, clearly shows that the intent of MoS metric is to create a false sense of safety.

B) A more basic and scientific fraud, also employed in this Q-RA, is when Q-RA's pretend a chemical can have more than one NOEL; i.e. more than one non-cancer 'no-effect' exposure level! (But because cancer may begin from a single DNA mutation, it's risks are legitimately calculated separately). This particular Q-RA goes to great length to find in the scientific literature, for each herbicide looked at, 'systemic' NOEL (i.e. all chronic effects observable at a gross (visual or microscopic) level of observation) "NOEL" and a reproductive effect "NOEL". The point of finding a chemical's actual NOEL is to protect from *any* non-cancer chronic effect of the chemical. Listing multiple NOELs is non-sense, but it has the advantage of implying that the lower NOEL is not the real NOEL. A naive risk manager such as an agency bureaucrat might well think: "Hmnn--maybe the safe level of exposure is the average of the two...". In fact, listing multiple NOELs is an invitation to risk managers and policy makers to dismiss the putatively real, more sensitive NOEL. For example (as occurs several times in this Q-RA), say that an exposure estimate results in exceedance of one putatively true "NOEL" but not the other. Will a risk manager or policy maker--looking only for conclusions, as she is unfamiliar with the Q-RA methodology--believe that the herbicide's risk is only half as bad because only one of two "NOELs" was exceeded? Or will they understand that exceedance of the lowest "NOEL" is the only valid way to assess risk?

It is ridiculously easy to find fatal inconsistencies between different Q-RA's NOELs for a given chemical. This Q-RA engages in an admirably extensive hazard analysis (literature search). Even had it done the proper thing i.e. only used the lowest NOEL found for every herbicide, that NOEL must obviously be the lowest in *all* the valid literature to be the real NOEL. There are so many parties performing chronic effect tests, with so many protocols and data gaps (the testing of

so few doses and end-points), that it is almost always impossible to know whether a stated NOEL is in fact the actual NOEL. Below, in our critique of the individual Q-RAs relied on by this FEIS, are several examples of this problem.

This Q-RA's admirable hazard analysis also shows just how cursory the examination of the test animal and its organs often is in a NOEL chronic effects test. Many, possibly a majority of the 'systemic' effects "NOELs" in this Q-RA are derived using protocols established before biochemistry was a full-fledged field, so those protocols may not even involve microscopic examination of all or any tissues, much less biochemical analysis. So this is another absurd basis on which to claim a true "NOEL".

In any case, as described in our general critique of Q-RA, chronic toxicity tests for a NOEL only test unrealistically high doses; so even the lowest "NOEL" is almost never the true NOEL. Q-RA's failure to test at many, including realistic, dose levels is one of its key frauds.

C) Toxicology data gaps--none of the following risks are estimated in this alleged risk assessment:

- mutagenicity (see p. ES- 7)
- Immune disruption (see p. ES- 8);
- Neurotoxicity (see p. ES-8);
- Carcinogenicity of 13 of the 21 herbicides (see p. ES-5);

- The toxicity of the impurities and added ingredients of herbicides (e.g. dioxins, HCB, nitrosamines; very potent carcinogens inter alia, and discussed elsewhere). Pesticide safety testing is rarely done on the product as sold, and is done on just the one "active" ingredient (with minor exceptions). While some contaminants have their safety discussed in this Q-RA (see p. III-C-36-89), their risk is not evaluated.

- Inhalation exposure was ignored for the public (not for herbicide workers), despite that scenarios such as early re-entry on public lands obviously occur (see p. ES-3). See our critique of herbicide drift safety.

- Synergistic exposures. All Q-RAs are forced to ignore this risk because to study them would take centuries, cost huge sums. It has been said to test all chemicals in commerce (c. 100,000) for synergy would require more test animals than there are stars in the known universe (see our Q-RA critique on synergistic effects). This Q-RA discusses these potential risks and the near total lack of data. Our cumulative exposures to many of these chemicals are just beginning to be quantitatively considered, and no Q-RA can evaluate this risk. Contrary to this Q-RA's claim (p. ES-8), synergism is found at significant rates in the occasions it has been looked for (once again, refer to our previous discussion on synergistic effects), perhaps not surprising given the subtlety of biology.

- Data gaps in general. This Q-RA acknowledges that its quantitative risk evaluation is weakened by large toxicology data gaps, and it includes a relatively thorough literature search description of the known toxicologic health effects (p. ES-7 & 8, II-C-1 to 35, III-C-36 to 89 and III-E-39 to 44). But while the literature search for these non-quantified health risks was thorough, the claimed qualitative (narrative) evaluation of risks from the literature, for effects that are below the chosen NOELs that are quantitatively evaluated, is missing. On none of the pages cited above is a narrative assessment of risk attempted for these other health effects found in the literature. This is tantamount to just ignoring (as many Q-RA do) these potential risks. That is because the risks that are quantified are done so in the natural sequence of a Q-RA, culminating in the statement of risks, which becomes the focus of the document. It seems unlikely that users of this Q-RA, who want to know estimated risks, will pay much attention to scattered narrative descriptions of additional potential health risks, when this Q-RA does not try to evaluate the magnitude of these risks.

D) Questionable findings of herbicide safety tests and estimated risks:

- People are animals, a part of the biosphere. Are risks really being assessed--more importantly does it fulfill NEPA--to almost ignore all effects short of death on all non-human organisms (p. ES-10)? Why is it that agencies are still using the lethal dose or concentration for 50% of a test population of animals (the LD-50 and LC-50) as the base from which to calculate a 'safe' or a 'no-effects' allowable level of a chemical? At least for humans we are working towards finding NOELs. If the resources used by ecological scientists on such tests in past decades had been spent on studying more subtle but still serious effects (such as reproductive failure, effects on infection, etc.), we would be a lot closer to analyzing the ecologic consequences of actions which NEPA mandates. Instead, the resource protection agencies throw in an safety factor literally picked out of thin air--typically 1,000-times less than the alleged NOEL--and assume the populations will be healthy at that exposure level, without any observation.

Possibly as bad, in this Q-RA, only aerial applications have even that minimal risk to wildlife and aquatic species

estimated. Ground based applications; even high pressure, vehicle-mounted nozzles (also common due to economic pressure of covering large areas of public land) are assumed to pose zero risk to anything, from an alga or microbe to a tree or mammal--other than a human (p. ES-11).

- Seven of the 21 herbicides have no RfD established (see p. III-C-36-89). Instead, a NOEL is used (which, as we describe above, lends itself to the MoS method of understating risks; and which, per the examples below, may not be the true NOELs).

- Similarly, four known 'other' undisclosed ingredients in herbicide formulations (solvents, dispersants, etc.)--known to be hazardous, whose risk was analyzed in this Q-RA--clearly do not have a NOEL, and thus no RfD (see p. III-C-90 to 94). Yet this Q-RA pretends they do by going ahead and evaluating their risk. Why pretend to be evaluating risk when a chemical's NOEL is unknown? Here, false "NOELs" are created from the lethal concentrations (plus a completely arbitrary safety factor); or from studies whose doses clearly aren't NOELs (see p. III-C-90 to 94 again). Ironically, the RfD process requires additional safety factors (guesses) when a NOEL is unknown or a NOEL study has deficiencies. How insane is it for anyone to allocate safety factors when they don't even know the NOEL onto which they are being factored?!

- Amitrole is far and away the most toxic (of known effects) herbicide--estimated to cause 1.4 tumors for every mg/kg body weight/day chronic dose (p. III-E-3), and with a World Health Organization Acceptable Daily Intake (ADI, equivalent of an RfD) of 0.00003 mg/kg bw/d for known non-cancer effects (p. III-C-36). If the standard safety factor of 100-fold was used to set the ADI, the ADI's NOEL would have been 0.003 mg/kg bw/d. But once again, rather than chose the apparent lowest NOEL, this Q-RA uses one that falsely shows greater safety: 0.025 mg/kg bw/d. This results in roughly eight times less estimated risk.

Pesticide worker risks are the only ones presented in any numerical metric (though a highly derivative one, whose risk cannot be critiqued): the amount of a herbicide that can be safely applied each day by a worker. Thus we can at least check the relative risks to workers of the different herbicides. Why is the calculated safe amount of amitrole for a pesticide applicator (e.g. 1.3 lbs/ day for a backpack applicator, Tables III-E-21 to 25) in the middle range of the other herbicides, when amitrole's cancer and non-cancer risk is at least about two orders of magnitude higher than any other of the 21 herbicides?!?

This discrepancy accents the vulnerability of Q-RA modeling to data diddling (GIGO: garbage in, garbage out). The exposure scenario estimates (with their multitude of quantitative variables involved in calculating the final estimate of how much of the chemical is taken in, from its initial release), being pure mathematical terms, are the ideal place to hide data manipulation. We would like to question why amitrole's first Q-RA step (hazard analysis--i.e. literature review) shows great potential risk; but the third and final step (risk analysis) shows no greater risk than less risky herbicides ... but we cannot, because no authors reveal themselves, and their data is also missing![542]

- Neurologic effects. Only five of the 21 herbicides are said to exhibit neurotoxicity, and neurotoxicity is not part of the quantitative risk evaluation. But as shown in our section on pesticide registration, pesticide neurotoxicity tests are rudimentary. As with so many other health effects, this Q-RA simply discusses this risk without evaluating it.

- Carcinogenicity. Thirteen of the 21 herbicides are not evaluated for cancer risk because they are said not to have had a cancer potency factor established. Yet for seven of these 13 we found independent (govt. agency or peer-reviewed) evidence of carcinogenicity, plus an eighth (clopyralid) which is in use without its carcinogenicity being evaluated. The other five we did not search the literature for carcinogenicity data--but given our success ratio for the others, it seems likely we would have found some evidence of carcinogenicity for most of these five. For the evidence of carcinogenicity we found, see our critique below of this FEIS' summary table of chronic health effects (which critiques 11 of the same 21 herbicides). In several instances, it is the contaminants in the herbicides a.i. that is a potent carcinogen. As with so many other effects, this Q-RA fails to evaluate significant indications of risk of this health effect.

- Immune effects. Immunotoxicity is also not part of the quantitative risk evaluation. As with so many other health effects, this Q-RA simply discusses this risk without evaluating it. But as shown in our critique of the idea that pesticide registration warranties safety, pesticide immunotoxicity tests are rudimentary, if they exist at all. The Q-RA states that the dermal reaction test is the most common immune test, yet the immune system is at work in every organ, system and cell.

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[542] A classic example of Q-RA data-diddling comes from Prof. Paul Connett at St. Lawrence U. in N.Y., in a waste incinerator Q-RA for dioxin emissions (showing no risk) that he critiqued. The exposure pathway model was discovered to have a 'deflection' variable set at 0.99 (range, 0-1.0), meaning that almost none of the dioxin molecules emitted from the stack fell to the ground, to enter the dominant pathway of human dioxin exposure (grazing by animals, rather than direct inhalation). Almost all the dioxins magically hover off the ground, never getting into any organism!

In fact inflammation is the specific and eponymous name for what occurs when the immune system is activated to kill off a foreign agent (with cell-lysing agents, macrophages and reactive oxidants). Obviously, this balance between 'self' and 'non-self' is delicate--it can easily tilt to auto-immune disease (the immune attacking self-tissue), or to inappropriate shut-down of the immune response. Any number of diseases, whether simple auto-immune reactions or not (including some cancers) are caused by, involve (or even cause) inflammation; i.e. immune activation. Moreover, many foreign agents are known depressors of the immune response, leading inter alia to deadly infections.[543] Last, another important function of immune activation is to create antibodies, tags that identify specific foreign agents (antigens), so that the next time the immune system encounters the antigen, it can mount its inflammatory attack sooner, i.e. at a much lower level of exposure to the foreign agent. This is what happens when an allergy develops; and it is one of the basis of chemical sensitivity--quite simply, chemical allergy. As they're foreign agents, it's likely that all pesticides licit some immune response. Certainly many, many have been so documented--see the thorough review of Repetto

- We disagree somewhat with the extensive discussion of mutagenicity risks in chapter III. For example, we reviewed the literature for mutagenicity claims of the 11 herbicides mentioned by this FEIS. All 11 tested positive for mutagenicity--almost all were positive in several or all mutagenicity tests--despite the FEIS's claims to the contrary for many. Those same 11 herbicides, popular with the USFS, plus 10 more reviewed in this Q-RA (that we did not critique) have their mutagenicity reviewed in this Q-RA. Because any valid scientific study with a positive result is of intrinsically greater value than a negative result, clearly this Q-RA's conclusion that mutagenicity is negative for all but four herbicides (p. ES-7) is wrong, given that its tables summarizing mutagenicity results (pgs. III-C-4 and 5) list positive results for almost every herbicide. Even a positive test in one species or in one type of cell, accompanied by several negative results, is of concern, because DNA is the origin of all biologic activity (mutagenicity means damage to DNA). This Q-RA extensively discusses the relation of mutagenicity and cancers, but it is more important to remember that genes regulate all biology, not just cancer. (Neither are all cancers caused by mutation to DNA, but that's another story).

-The NOEL used for imazapyr's safe dose estimate is 500 mg/kg b.w./day. But the Herbicide Handbook (7th Ed., Weed Society of America, 1994) lists a NOEL of 150-175 mg/kg bw/d. Obviously the former is not the NOEL; the latter is, unless there is an even lower NOEL. Therefore of its non-cancer health effects evaluated, imazapyr's risks were underestimated at least 3-fold (and it's non-carcinogenicity test result is questionable, as explained elsewhere).

It's interesting to note that a herbicide with an all but identical structure, imazapic (not imazapyr), has a RfD of just 0.05 mg/kg b.w./day, according its manufacturer BASF (see table IV-16, p. IV-50 & 51 of the LNF BGWR&BAWM FEIS). Thus its NOEL would be 5 mg/kg b.w./day (i.e. taking away an RfD's standard 100-fold safety factor). That is 100 times lower than the look-alike imazapyr molecule's already questionable NOEL.

E) This Q-RA analyzes the fire-volatilized herbicide risks of the 'brown-&-burn' land management technique--getting rid of unwanted vegetation by broadly killing vegetation with herbicides, then setting fire to the area (though ignoring any toxic combustion products of burning herbicides). This method may be within the scope of this BDNF weed control EIS, considering its central purpose of aggressive weed control. For these volatilized inhalation risks, this Q-RA uses, instead of the (incomplete, but best available) 'safe dose' standards, the most fraudulent ones that exist: the Threshold Limit Values (TLVs) of the American Conference of Governmental Industrial Hygienists (ACGIH)'s TLV Committee. Despite its carefully chosen name, the ACGIH is and always has been a chemical industry-dominated group whose TLV Committee for decades has secretively established TLVs--highly influential, allegedly safe limits for airborne toxins in the workplace. It has been conclusively established that:[544]

1) many--perhaps most, until recently--TLVs were not set based on their known risks; but simply on what the chemical's chief user or manufacturer wanted them to be (sometimes, whatever the chemical's concentration on the factory floor was!), but always higher than real health-based values such as NIOSH's; and

2) the ACGIH's TLV Committee continues to be dominated by incorporated chemical manufacturers (whose legal obligation is to make as much profit as possible) and their allies; and it continues to refuse to set TLVs in an open process that can be reviewed by outsiders and peers. Unfortunately, TLVs are hugely influential--many governments, including sometimes the U.S.A's OSHA, have adopted them as safe workplace--even general 24 hr exposure--limits. Thus this part of this Q-RA should be thrown out.

Regarding the separate risk of fire smoke from prescribed burns (p. II-6 to II-12; i.e. not the risk of herbicides carried in the smoke), it is as always assumed that a narrow analysis of the many risk factors in smoke and the many health

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[543] Repetto & Baliga 1996 'Pesticides & the Immune System: public health risks' World Resources Institute Wash. DC.

[544] S. Roach, S. Rappapport, B. Castleman and G. Zeim, in a series of papers (together, in pairs and alone; one of the later, with references to others is: S. Rappaport 1993 'Threshold Limit Values, Permissible Exposure Limits, and Feasibility...', Amer. J. Ind. Medicine 23:4:683-694)

endpoints constitutes an valid assessment of risks. It is true that the cancer risk of the potent carcinogenic poly-aromatic hydrocarbons (PAHs, basically creosote) is one of the main risks of smoke exposure, but by no means is it the only one. A new paper presents a sobering estimate of just the risk of mortality from inhalation of very low concentrations of small (deeply-respirable) particulate matter (i.e. PM2.5--2.5 microns and smaller in diameter), which this Q-RA acknowledges is the main component of smoke. For every increase of 10 micrograms of PM 2.5 in a cubic meter of air (10 ug/M3) inhaled there is a 1.5% increase in mortality. That increased risk held over the entire range of concentrations that is typical in cities (0 to 35 ug/M3)--there was no dose threshold below which there is no increase in mortality, as industries emitting PM2.5 have alleged.[545] This risk estimate held across six large U.S. cities, and it validated the risk found in a previous large study. As the authors state, there is a large body of literature supporting the mechanisms of PM2.5 biologic effects, many of which are initiated by the immune activation from their respiration. Thus it is certain that many effects beside mortality result from PM2.5 exposure. However there are many components in smoke; some are part of the particle, some adhere to the surface, and some are in the gas phase; all are with known, and certainly all have additional unknown, toxicities. Again, only the cancer risks of PAH in fire smoke are analyzed here. This enables the false claim that the risk of prescribed burn smoke is negligible or small and manageable (p. II-10).

CONCLUSION:

Despite these massive data gaps and unwarranted assumptions of safety, characteristic of Q-RAs in general, this Q-RA concludes (as do the other Q-RA's that this FEIS cites) that a significant minority of the many exposure scenarios evaluated exceed the minimal risk thresholds. Also in these USFS-commissioned or USFS-used Q-RA, a few of the herbicide estimated risks are significantly higher than the others. Adding these acknowledged risks in these Q-RA's to the data gaps and known risks which we have shown, it is clear that NEPA's mandate to analyze and mitigate impacts has not been met.

It telling to inquire why on the one hand Q-RAs pretend that large data gaps and significant faulty assumptions can quantified in a risk estimate; while on the other hand--for those exposures that it estimates have serious or non-trivial risk, a risk manager using a Q-RA to help write an EIS always guarantees interested parties that certain absolutely *unquantified* mitigation measures will reduce the risk to below significance. The mitigation measures' effects are always vague; never to any germane degree quantified, eschewing even a revision of the measure's effect on the Q-RA's already-calculated numerical risk estimate. To validly assess environmental impacts in an EIS, either the guaranteed risk reductions must be shown to a reasonable degree, which inevitably requires quantification; or the numerical risk estimates must be admitted to be worthless as a reasonable estimate of risk. We believe science's immense data gaps, and the faulty assumptions of Q-RA demonstrate that the latter is the only approach to an honest impact and risk assessment that will work. Especially as these faulty assumptions and data gaps concern a biosphere that is obviously subtle and vulnerable, a more precautionary approach to environmental policy is irreducibly indicated.

<u>USFS EIS' ANALYSIS OF HUMAN HEALTH CONSEQUENCES OF HERBICIDE USE</u> (APPARENTLY THEY RELY ON INFORMATION VENTURES Inc.'s FACTSHEETS ON HERBICIDE RISK)

The single place this FEIS makes any specific claim about crucial question of chronic toxicity is summary Table 3.5.3.[546] This table uses one or two-word phrases to summarize just four of the dozens of major potential chronic health effects, for just eleven of the unlimited number of herbicides to be used. Incredibly (or perhaps of necessity, given how wrong they are) these claims are un-cited. A reader must guess, from text in Chapters 2 & 3, what is the source.[547] However, the text hints, and in fact the BDNF confirmed, in response to a FOIA inquiry[548] , that the table's source is a consulting company (Information Ventures Inc.), contracted to provide the USFS with factsheets summaries of pesticide safety and risks. These pesticide factsheets have a section on chronic effects that always evaluates with a short phrase, just four

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[545] J. Schwartz et al. 2002 'The Concentration-Response Relation Between PM2.5 and Daily Deaths' Env. Health Perspectives:110:10:1025-29.

[546] Note that the preceding FEIS table (3.5.2, summarizing *acute* toxicity values) is of little value in an FEIS' analysis of consequences, because chronic exposures are ubiquitous and acute exposures rare, while chronic effects are often as serious but less reversible than acute effects. Short of a spill or an applicator's accidental exposure, the potential consequences that an EIS should evaluate carefully are the lower but ubiquitous exposures that can lead to chronic effects.

[547] For some strange reason, or from incompetence, this table and relevant text are not in the 'Potential Consequences' chapter 4, but in the preceding chapter 3 on 'Existing Conditions'.

[548] **[NEED THE FOIA RESPONSE CITE HERE] <<<<==**

effect categories--the same four as in the FEIS' table,[549] and in the same order as the table. It appears that the USFS simply further summarized the chronic toxicity phrases in the factsheets into a single word to create Table 3.5.3--especially since the USFS has told us that the table's source is the Information Ventures factsheets.

[** NEED THE CITATION FOR THIS FOIA REQUEST THAT RYAN MADE]
According to the USFS's response to the FOIA request, the sources for the two health effects summary tables and for some unspecified related text are:

-Information Ventures, Inc. pesticide factsheets prepared for the USFS
- [** NEED TO FILL IN REST OF THIS LIST FROM INFO RYAN GOT, BUT I THINK I'VE COVERED THEM ALL IN ONE PLACE OR ANOTHER]

Also incredibly, considering that this table is the FEIS' only attempt to evaluate herbicide chronic health effects to humans or other organisms, Information Ventures' factsheets, prepared for the USFS, contain not a single citation. In peer-reviewed publications, such unsubstantiated claims would be summarily dismissed--i.e. not published. We attempted to uncover the authorities the Information Ventures pesticide factsheets rely on to make claims on chronic toxicity, because this FEIS relies completely on these critical claims, without bothering to find out if they are credible.

When asked, Information Ventures listed the following sources of information in their un-cited factsheets:[550]

- unknown USFS documents;
- pesticide labels & registration materials;
- Material Safety Data Sheets;
- WHO 'Env. Health Criteria Series' monographs;
- a "Piedmont area" Q-RA;
- the Syracuse Env. Risk Assmnt. (SERA) Corp.'s pesticide Q-RA for the USFS;
- a Labbatt-Anderson (env. consultants) series of pesticide factsheets or more extensive assessments;

Perhaps the unspecified USFS pesticide documents are what the USFS has on its pesticides web page, which in turn contains SERA pesticide Q-RA and Information Ventures pesticide factsheets for information on pesticide risks. The labels & registration and the SERA Q-RA are critiqued in other sections. The 'Piedmont area' and the Labbatt-Anderson information were not available to our Information Ventures contact at the time of our personal communication. Given the numerous sources of herbicide information that we have reviewed, we would expect theme to contain only the much-recycled information originally generated during each herbicides registration.

MSDS contain no information that is not available in other sources reviewed by plaintiffs, such as the requirement to list known carcinogens. They need only list the hazards of listed ingredients, i.e. for pesticides, only the a.i. They almost exclusively contain information to protects workers from acute toxic risks and other acute hazards such as explosions, asphyxiation's, etc.[551] Even the information on permissible inhalation limits and Threshold Limit Values (PELs & TLVs) reflect acute risks only;[552] except for a few OSHA PELs, whose chronic risks are, again, already reviewed here.

Finally, the World Health Organization's (WHO) Env. Health Criteria (EHC) chemical monographs are written for and found on the web page of the Int'l Program on Chemical Safety (IPCS--a joint chemical safety program of WHO, UNEP & the ILO). A perusal of the list of monographs revealed only one herbicide, 2,4-D. It actually has two EHCs (unlike most chemicals on the list of EHCs), one for ecological and one for health effects. As monographs, they are thorough descriptions of the literature available at the time they are prepared, in this case 1984 (health effects) and 1989 (ecological effects). Thus, despite being thorough, they are very outdated for a high use and dangerous chemical as 2,4-D. The government and peer-reviewed published literature that we summarize for 2,4-D is in no way refuted by these EHC monographs. In fact, they contain much additional information indicating at least some risks at concentrations generally encountered.

However, the scope (four chronic effects) of the FEIS' chronic toxicity table and the Information Venture factsheets' section on chronic toxicity is identical to the scope of FIFRA's requirements for chronic toxicity testing--other than a fifth chronic toxicity test, but that one would tend to be dropped in summaries because it is not easily summarized (the general chronic toxicity test, it looks for chronic effects in several organs). Therefore we are confident that Information Ventures

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[549] Except that "developmental" in the factsheets is changed to "teratogenic" (i.e. causing birth defects) in the FEIS' table. Likely the headings are equivalent, as developmental toxicity tests dose a pregnant animal to look for birth defects in fetuses (see C. Klaasen (ed.) 2001).

[550] Personal communication late Nov. 2002 Bill Thompson, Information Ventures; to Tony Tweedale, Missoula MT.

[551] American Chemical Society 1990 'Informing Workers of Chemical Hazards: the OSHA hazard communication standard', Wash. DC

[552] Roach, Rappaport et al.

used (at least in large part) EPA's pesticide registration data when it summarized the chronic toxicity of each pesticide it has factsheets for. This is corroborated, for example, by the EPA Picloram Re-registration Eligibility Document (RED) summary,[553] whose summary of picloram's chronic toxicity during its re-registration matches the FEIS's table and matches Information Venture's picloram factsheet.[554]

Information Ventures says they are not experts in advising or researching pesticide risks, and for this reason they say they had their draft pesticide factsheets for the USFS reviewed by toxicologists[555] No board-certified toxicologist should approve a summary of toxicity information that fails to make a single citation to back up its claims!

To indicate the complete failure of the Information Ventures pesticide factsheets and the FEIS' table to represent chronic toxicity risks, plaintiffs will now rebut nearly every claim of chronic exposure safety (in most cases with multiple citations to the independent literature) that is made in this summary table, by using the independent literature (either peer reviewed published or government agency data). It is very important to keep in mind, however, that the USFS and Information Ventures chose to ignore everything but four chronic health end-points, and for just 11 herbicides (i.e. 44 claims). It should be obvious from the amount of independent literature available to rebut these 44 claims, that as much independent literature exists to rebut any future claims of safety that the USFS attempts to make about other chronic health end-points (including for every single herbicide registered, which this FEIS approved for use on the BDNF).
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Another series of herbicide quantitative risk assessments (Q-RA) are cited to back up claims of no unreasonable consequences by the LNF's BGWR&BAWM FEIS, which in turn was incorporated by this FEIS. These Q-RAs were done for the USFS under contract by Syracuse Env. Research Associates (SERA). We thoroughly reviewed the heart (the quantitative modeling of exposure and risk) of two of them: picloram and 2,4-D. We also quickly scanned the basics of several of the other herbicide Q-RAs--their hazard, exposure & risk analysis. These have at least some of the same flaws as the two that we closely critiqued have. As all of them have a unified format, we predict that they all have the same crucial flaws as the picloram and 2,4-D ones, to wit:

EPA's RfD for picloram is 0.07 mg/kg of body weight per day. But this Q-RA that the USFS is relying on uses a picloram RfD almost 3 times higher (less safe), 0.2 mg/kg b.w./day (which happens to be the same as a the manufacturer's, Dow's, calculated RfD, which EPA accepted in setting the level of picloram allowed in food (food tolerances). It's definitely not the regular, EPA-approved picloram RfD found in EPA's Integrated Risk Information System (IRIS) database. Even so there are a few modeled exposures in this Q-RA (which models exposures of pesticide applicators, the general human population, aquatic and terrestrial organism) that exceed the more lenient RfD. How many more would exceed it if they had used to stricter RfD?

Last, picloram always has hexachlorobenzene (HCB) contamination, a potent carcinogen and with a very low RfD for other health effects, as already described. As usual in Q-RA's, this risk is not estimated. It has been shown that wildlife (just as EPA calculated for human picloram applicators) are at great risk of cancer or other disease from HCB. Predators of ungulates eating only picloram-sprayed forage were, in a local Environmental Assessment (EA), calculated to ingest 0.021 to 0.034 mg/kg per daily meal of HCB--up to 43 times the HCB non-cancer 'safe dose' (RfD) of 0.0008 mg/kg/d. The ungulates were claimed to ingest less than the HCB safe dose, without any quantification; and the Finding of No Significant Impact (FONSI) to avoid an EIS was finalized![556]

The 2,4-D SERA Q-RA uses the correct RfD. Consequently, a significant number of the exposure pathways are modeled to exceed that safe dose. However 2,4-D is being re-evaluated by EPA under FIFRA's Special Review procedure, due to concerns of greater toxicity. This herbicide too has potent, bioaccumulative impurities, the dioxins, as described elsewhere here.

These SERA Q-RAs seem to ignore cancer risk, as they apparently just use a herbicide's RfD to evaluate risk.

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[553] EPA/OPP 1996 (Picloram RED factsheet).

[554] Except the conclusion for mutagenicity is changed from EPA's "no evidence of mutagenicity".

[555] Personal communication late Nov. 2002.

[556] U. of Montana May 1998.

DRIFT

EXTENT OF DRIFT, ESPECIALLY FROM AERIAL APPLICATION:

[After the BDNF Weed FEIS I had expressed my concern to the EIS contact, Leaf Magnussen about aerial spraying, along with a study indicating the risk; she later left me a tel. message saying that the droplet sizes are 40 times larger than the study I had given her (re: Bt drift, below). Her response fails to respond to the following hard data:].

While it seems to be true, as the Regional Office claimed in their response to our BNF appeal, that aerial herbicide formulations are generally released at larger droplet sizes than are aerial insecticide formulations; the difference is small enough that there is significant overlap:

> "Recommended droplet sizes for fungicides, insecticides and herbicides are 150-250, 200-300 and 250-400 microns, respectively."[557]

Even the pesticide industry has concluded that most nozzle sizes and designs result in droplets smaller than 150 microns (and that some here-unstated formulations cause significant drift).[558]

Critically, it has been found that the peak ambient air concentration of 45% of agricultural pesticides is eight to 24 hours post-application; whereas most measurement of drift is made long before, leading to severe underestimates and inadequate mitigation of drift.[559]

Ignoring that factor, it is also documented that evaporation shrinks aerially released pesticide formulation drops as they descend, and that the vortexes of the aircraft break up drops; both factors that lead to greater drift than planned or modelled.[560] In addition, spraying during the no-wind condition of a temperature inversion will prevent much (the fine fraction) of an application from falling to the ground at all (normally sun-heated air cools as it rises, then falls: i.e. it mixes; but as cool equals dense air, a lack of insolation causes stratification). After external winds or the sun break an inversion, drift or evaporation resumes.

The National Academy of Sciences says that from 5% to well over 60% of an aerial application will drift off-target, depending on weather conditions, notably wind and temperature inversions (where cold dense air near the ground retards pesticide molecules from falling to the ground, to be dispersed as the inversion breaks and the cold air warms & rises).[561] Typically, 1% of an application reaches the direct target pest (e.g., weed), while 40% leaves the general target area.[562] Another estimate is that less than 0.1% of pesticides ever reach their target pests.[563] Also, it has been calculated that 20% of a pesticide application on surfaces volatilizes.[564] A 20 micron drop released just 10 feet off the ground will drift 1,056 feet in just a 3 mph breeze.[565] The fine spray typical of ultra-low volume application methods (median drop diameter <200 micrometers) increases off-target drift *ten-fold* over conventional nozzles producing median drop diameters around 300 um.[566] A field experiment acounting for all the main variables for drift from **ground-applied** pesticides (wind, nozzle diameter and pattern, boom height, pressure and spray shielding) found that when nozzle diameter was decreased (to simulate more economical and swifter applications), but a shield was added to compensate for increased drift; drift nontheless increased by 29%.[567]

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557 OH State U. Cooperative Extension Service 1992 'Reducing Spray Drift' Extension Bulletin #816; pg. 5.

558 A. Maciorowski 24 Mar 1994 'Qualitiative Assessment of SU Herbicides' US EPA Memorandum to Evert Byrington of Science Analysis & Coordination Branch Chief , EFED Div., OPP.

559 Pesticide Action Network N. America (PANNA) 2003 'Secondhand Pesticides: Airborne Pesticide Drift in Calif.' accessed June 2003 at: http://panna.org/resources/documents/secondhandDriftAvail.dv.html

560 Teschke et al. Jan. 2001 'Spatial & Temporal Distribution of Airborne Bacillus thurungentius...' Env. Health Perspectives:109:47-52.

561 Ntl. Academy of Sciences/National Research Council/Board on Agriculture/Committee on Long-Range Soil and Water Conservation 1993 'Soil & Water quality: an agenda for agriculture' Wash. DC: Ntl. Academy Press. p 323-4.

562 U.S. Congress Office of Technology Assessment 1990 'Beneath the bottom line: agricultural approaches to reduce agrichemical contamination of groundwater' Report No. OTA-4-418. Washington DC: U.S. Government Printing Office.

563 D. Pimentel April 1999. Speech at NCAMP/Beyond Pesticidesannual conference, Boulder CO (Dr. Pimentel is a noted researcher on pesticide econimics and resistance at Cornell Univ.).

564 Dr. Alan Cessna , Env. Canada/Ntl. Water Research Institute Winter 2001 letter to the J. of Pesticide Reform.

565 Ohio Cooperative Extension Services Bulletin # 816, on pesticide drift.

566 S. Bird et al 1996 'Off-Target Deposition of Pesticides From Agricultural Aerial Spray Application' J. Env. Quality:25:5:1095-1104.

567 T. Wolf. et al. 1993 Oct. 'Effect of protective shields on drift and deposition characteristics of field sprayers' Canadian J. Plant Science:73:1261-73.

So the obvious incentive to make significant cost savings on both product expense and expensive flight or ground-vehicle time by covering the target area more quickly via a smaller nozzle diameter (smaller released drops) results in much greater drift. The EIS's fail to state what nozzle size & nozzle fan angle limits they will use, nor do they specify the drift reduction agents or their effectiveness. Nor may drift cards indicate drift if the vortex of the aircraft has sent the drift over the top of the drift cards. Nor may attempting to limit aerial spraying to periods of less than 6 mph winds prevent significant drift. All this is adequately shown by the studies cited here.

Even the industry (NACA) Spray Drift Task Force's vaunted AgDrift model cannot be validated with field data when the distances to be calculated are not near, causing *a hundred-fold* under-prediction of drift at far distances when the release is from any helicopter scenario.[568] This shocking underestimate of drift may be due to the persistence of helicopter vortexes, which have been calculated to influence airborne pesticides for over a minute; far beyond three hundred feet (a typical "conservative" buffer zone in aerial applications) in a typical 4.47 m/s wind . It may also be due to evaporation, which has been calculated in the same model to be a significant cause of drift[569] (yet less volatile oily formulations lose this advantage by being lighter drops than aqueous formulations).

Of 16 studies on drift measurement found by one group, drift was detected no matter how far away they monitored, from 1.25 to 50 miles.[570] **As to herbicides (refuting the RO's above claim)**: 2,4-D drifted at least 50 miles from application, dicamba at least five to 10 miles, and paraquat at at least 20 miles.[571] At least three more studies document significant 2,4-D (and triallate) drift beyond target areas.[572] Rainwater concetrations of 2,4-D have exceeded allowable contaminant levels.[573] Even the U.S. EPA has estimated that 0.01% to 2% of several pesticides will drift as far as a mile, after actually measuring pproportionaly greater concentrations at a quarter-mile from the application field.[574] That is much more drift than users and even EPA registration typically assumes will happen!

A liquid Bt insecticide formulation drifted over one kilometer. Released at 110-125 microns diameter, these drift droplets dispersed to 4-7 microns diameter. This study noted they could find no literature validating lack of drift.[575] B.t.k. drift was documented up to 3,150 meters from a target spray area, concluding that "this biopesticide would likely drift farther than 3150 m under similar atmospheric and terrain conditions, given the level of detection observed at 3150 m." This study was done at wind speeds between 5.22 and 6.14 miles per hour.[576] Even the USDA's statement on pesticide drift control, used by the USFS, calls for aerial spraying only below 5 mph winds (but ignoring inversion drift).

Genetically engineered (GE) plant pollen and microbes offer the opportunity of a different, confirmatory metric of how far pesticides may drift: to measure the engineered gene in downwind native organisms (a type of "gene flow") . Recently it was found that GE pollen (of the first GE plant that easily hybridizes with native plants--creeping bentgrass; GE'd to be herbicide resistant, for golf courses) was taken up by native plants as far as 23 km (14 miles) downwind...far further than the previous documented record of 3 km![577] No suprise then that dangerous amounts of pesticides often drift much further in more easily airborne packets.

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[568] S. Bird et al 2002 'Evaluation of the AgDisp Aerial Spray Algorithms in the AgDrift Model' Env. Toxicol. & Chem.:21:3:672-681.

[569] M. Teske et al 2002 'AgDrift: A Model For Estimating Near-Field SPray Drift From Aerial APplications' Env. Toxicol. & Chem.:21:3:659-671

[570] C. Cox Spring 1995 'Indiscriminately From the Skies' J. Pesticide Reform:15:1:2-7 (citations #33-38).

[571] E. Robinson & L. Fox 1978 '2,4-D Herbicide in Central WA' J. of the Air Pollution Control Assoc.:28:10:1015-20; **and** P. Westra & H. Schwartz 1989 'Potential Herbicide Volatility & Drift Problems on Dry Beans' Service in Action, Colorado State U. Cooperative Extension; **and** C. Glantz et al. 1989 'An Assessment of the Meteorological Conditions Associated With Herbicide Drift...' Battelle Pacific NW Laboratories.

[572] F. Larney et al. 1999 'Herbicide transport on wind-eroded sediment' J Environ Qual:28:1412-1421 **and** D. Renne 1979 'Experimental studies of 2,4-D herbicide drift characteristics' Agric Meteorol:20:7-24 and D. Waite et al. 2002. Environmental concentrations of agricultural herbicides: 2,4-D and triallate' J Environ Qual:31:129-144.

[573] USGS 1997 'Pesticides in the Atmosphere' Factsheet FS-152-95. Sacramento, CA:U.S. Geological Survey. Available: http://ca.water.usgs.gov/pnsp/atmos [accessed 15 March 2002].

[574] A. Maciorowski 1994.

[575] Teschke et al. Jan. 2001.

[576] W. Whaley 1998 'Canyon Drift and Dispersion of Bacillus thuringiensis and Its Effects on Select Nontarget Lepidopterans in Utah' Env. Entomology:27:3:539-548.

[577] Lidia Wastrud et al. 5 Oct. 2004 'Evidence for Landscape-level, Pollen-mediated Gene Flow From Genetically Modified Creeping Bentgrass With CP4 EPSPS As a Marker' PNAS:101:40:14533-8.

EFFECTS OF DRIFT AND VOLATIZATION:

Before describing the literature that conslusively proves very significant human, ecologic and economic adverse effects from the drift and volatization of herbicides specifically, we wish to emphasize that few if any of these studies look for more than visible damage, which obviously is a small subset of the damage that posions can cause. However, a resonable number do look at chronic effects, which in the case of crops typically does not extend beyond one harvest.

States receive about 2,500 complaints about pesticide drift incidents a year.[578] However federal collection of any pesticide incidents was eliminated many years ago; and in any case it is obvious that far, far more drift incidents than that occur. California still attempts to keep records of pesticide injury; in 1991 they reported 20% of all pesticide injury reports were from over pesticide drift (350 drift injury reports, but pesticide injury overall was acknowledged to be under-reported).[579] More recently (1998-2000) drift accounted for 51% of reported pesticide injuries in California, possibly due in part to more & improved reporting to the system.[580] Obviously these are almost all limited to acute injury.

Many instances of **human** chemical injury from drift have been documented, despite the uncontrolled nature of this type of exposure that makes it difficult to know what agent has made one acutely ill. Children living a over a quarter mile from an orchard using ground-level blowers to apply pesticides had these pesticide metabolites in their bodies at levels 50% greater than the controls.[581] California Air Resource Board monitoring as far as 500 feet from the target found levels of at least three pesticides that significantly exceeded their estimated safe doses; even though they failed to account for many health end-points and did not allow for the vulnerability of children by adding a 10-fold safety factor.[582] Other documentations of human injury from pesticide drift exist,[583] including from herbicides (2,4-D and paraquat, both applications resulting in severe chronic injury).[584] 165 poor persons were acutely posisoned (respiratory symptoms persisting at least 11 days after exposure, without health insurance, no further medical follow-up occured) by the evaporation a quarter mile away of a soil fumigant; after it was injected 17-18" into soil, the soil was compacted, and weighted boards covered the 18 acres.[585] Some 35 girls and adults where taken to hospital after an"ultra-low volume" malathion insecticide application drifted to their nearby Saratoga Co., NY softball field in 2001; and ongoing effects lead to lawsuits.[586] Various studies document pesticide take-home and drift exposure and apparent health effects in the families of agriculture workers.[587]

As to **ecologic** damage, another study of Bt drift found that aerial applications of B.t/k. killed butterflies and moth species almost two miles (3000 meters) from a target spray area, concluding that "[t]he potential negative impact of drift on other B.thuringiensis-sensitive nontarget lepidopteran species can be significant even when their larvae are a considerable distance from the release site."[588] The potent sulfonurea (SU) and related ALS-inhibiting herbicides, used by the USFS, are famous for their ability to cause non-target plant and crop damage at incredibly low concentrations--i.e. far from the application point--see immediately below. A careful, large scale analysis of amphibian loss in California was strongly associated with pesticide drift from upwind agricultural use while habitat loss was less strongly associated; neither u.v. radiation or climate change patterns were asociated with amphibian loss patterns (and introduced predators & disease

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578 U.S. EPA 2001'Draft Pesticide Registration Notice (Spray and Drift Label Statements', accessed Dec. 2004, http://www.epa.gov/oppmsd1/PR_Notices/prdraft-spraydrift801.htm

579 California Environmental Protection Agency/Dpt. Pesticide Regulation/Worker Health and Safety Branch 1994. ‘Pesticide Illness Surveillance Program: Summary Report. Health and Safety Report’ HS-1692. Sacramento, CA.

580 M. Reeves et al. 2003 'Greater Risks, Fewer Rights: U.S. Farmworkers & Pesticides' Int'l J. Occup. & env. Health:9:30-39

581 R. Fenske et al. 2000 ‘Strategies for Assessing Children’s Organophophorous Pesticide Exposure in Agricultural Communities’ J. Exposure Analysis & Env. Epidemiology:10:662-671.

582 PANNA 2003.

583 C. Cox Spring 1995 'Indiscriminately...' (citations #2-8, 50-51).

584 C. Cox Spring 1995 'Indiscriminately...' (citations #4, 6 & 7).

585 Centers for Disease Control & Prevention 20 Aug. 2004 'Brief Report: Illness Associated with Drift of Chloropicrin Soil Fumigant into a Residential Area--Kern County, CA, 2003' Morbidity & Mortality Weekly Rpt.:53(32):740-742 (http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5332a4.htm).

586 A. DePalma 1 Sept. 2004 'When Pest Killers Make Mistakes' NYT, front pg. Metro sec.

587 B. Thompson et al. 2003 'Pesticide Take-home Pathway AMong Children of Agricultural Workers' J Occup & Env Med:45:42-; **and** R Fenske et al. 2000 'Strategies for Assessing Children's OP Pesticide Exposures in Agricultural Communities' Ann Env Epidem.:10:662-.

588 J. Barry et al. 1993 ‘Predicting and Measuring Drift of Bacillus thuringiensis Sprays’ Env. Toxicol. & Chemistry:12:1977-1989.

were not testable, but seem unlikely causes of such large scale amphibian loss).[589]

As to **economic** damage, the ALS inhibitors have already caused tens of millions of dollars of documented damage to crops, via drift, even though they are new.[590] In fact, following early (1989) and persistent problems in eastern WA state of crop damage from the long-range use of SU and other herbicides,[591] EPA's best pesticide environmental fate and effects scientists eventually (1994) recommended that ALS inhibitors be banned from aerial applications, that no new ones be registered for any use until analytical methods that can detect the concentrations known to cause this drift damage are developed(!), and that their use be reduced![592] Low cholrsulfuron levels (1/100th the label application rate) caused severe yield and growth inhibition in several taxonomically diverse crops, especially when applied at critical developmental stages.[593] Similar damage occured at even more tenous simulated SU drift (down to 0.33% of label application rate of a thifensulfuron/tribenuron mixed formula).[594] 1/500th the application rate of chlorsulfuron reduced cherry yields 85%, in the absence of visible damage; and *1/10,000* of its label rate damaged white mustard seed production![595]

Economic damage from drift of **traditional herbicides**. According to various Extension Service reports, glyphosate, 2,4-D, dicamaba, clopyralid and the ALS inhibitors (eg. the sulfon ureas) damage non-target crops, including from drift, when applied at labelled rates.[596] Endosulfan insecticide concentrations of 0.004 mg/L (4 ppb, if that's a concentration in water) severely damaged amphibian populations, 200 m away from an aerial application zone (at the allowed rate).[597] Sub-lethal applications of 2,4-D and dicamaba caused persistant crop loss in experiments.[598] Garlon 4, the most volatile of the triclopyr formulations, is suspected to have volitalized on a hot, windy day after a June 2004 application at a California state park, then drifted onto two adjacent vinyard properties, killing up to $500,000 worth of high-quality grapevines and olives.[599] That's a lot of dead plants from an incident that did not involve drift at the time of application! In the 1990's a team published at least five papers in Weed Technology on their experiments of aerial application of several of the herbicides the USFS is using, consistently finding severe and season-long crop damage (including cherry trees) even at 1/100th the maximum label application rate (to simulate quite long drift). They consistently found that chlorsulfuron (a SU), 2,4-D and glyphosate caused the most crop damage (the other herbicides tested were bromoxynil, thifensulfuron and tibenuron)[600] Further confirming that it's the USFS' herbicides that do the most damage to non-target vegetation, another team found that glyphosate caused significant damage to seven crops, compared with only one

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589 C. Davidson et al. Dec. 2002 'Spatial Tests of the Pesticide Drift, Habitat Destruction, UV-B, and Climate-Change Hypotheses for California Amphibian Declines' Conservation Biology:16:6:1588–601.

590 Idaho Dpt. Agriculture 18 Jan.. 2002. Press release & publications 'Idaho State Department of Agriculture completes Oust investigation'; **also** M. Ferullo 2002 'Farmers sue DuPont, seek compensation from Interior for Alleged Herbicide Damage' Chem. Reg. Rep. 26:553; **also** S. Turner 1987 'Post-application movement of sulfometuron methyl from treated rights of way areas via wind (soil) erosion' Proc. Fourth Symposium on Environmental Concerns in Rights-of-Way Management. October 25-28, 1987. Indianapolis, Indiana; **also** Fletcher 1993 **and** Burns 1999.

591 G. O'Neal 1989/ Apr 28. email re: The problem of undetectable residues of drifted herbicide causing non-target crop damage. [E-mail from Gary O'Neal, EPA Air & Toxics Division/Reg 10, to EPA's Anne Lindsay].

592 A. Maciorowski 1994.

593 J. Fletcher et al. 1996 'Potential impact of low levels of chlorsulfuron and other herbicides on growth and yield of nontarget plants' Environmental Toxicology & Chemistry:15:1189-96.

594 D Gealy et al. 1995 'Growth and yield of pea (*Pisum sativum* L.) and lentil (*Lens culinaris* L.) sprayed with low rates of sulfonylurea and phenoxy herbicides. Weed Science:43:640-47.

595 A. Maciorowski 1994.

596 J. VanDyk Last updated 7/12/1999 'Drift injury to corn & soybean' http://www.ipm.iastate.edu/ipm/icm/1997/6-16-1997/driftinj.html (accessed late 2003).

597 M. Berrill et al. 1998 'Toxicity of Endosulfan to Aquatic Stages of Anuran Amphibians' Env. Toxicol. & Chem.:17:9:1738-1744.

598 J. Gilreath et al. 2001 Jul. 'Crop injury from sublethal rates of herbicide. Ii. Cucumber. Hortscience 36:674-76 **and** '...Iii. Pepper', pp. 677-81.

599 Beyond Pesticides , 9 July 2004 'Vintners Blame Pesticides For Damage' Daily News, originally reported in Wine Spectator: http://www.winespectator.com/Wine/Daily/News/0,1145,2528,00.html).

600 Al-Khatib K, Mink GI, Reisenauer G, Parker R, Westberg H, Lamb B. 1993 'Development of a biologically-based system for detection and tracking of airborne herbicides' Weed Technology:7:404-10; **and** Alkhatib K, Parker R, Fuerst EP. 1992 'Alfalfa (medicago-sativa) response to simulated herbicide spray drift' Weed Technology:6:956-60; **and** Alkhatib K, Parker R, Fuerst EP. 1992 'Sweet cherry (prunus-avium) response to simulated drift from selected herbicides' Weed Technology:6:975-9; **and** Bhatti MA, Alkhatib K, Parker R. 1996 'Wine grape (vitis vinifera) response to repeated exposure of selected sulfonylurea herbicides and 2,4-d' Weed Technology:10:951-6; **and** Bhatti MA, Alkhatib K, Parker R. 1997 'Wine grape (vitis vinifera) response to fall exposure of simulated drift from selected herbicides' Weed Technology:11:532-6.

species for MCPA and mecoprop, and asulam was the least toxic (they modeled that vaporization after glyphosate application should not cause that damage, implying that drift would).[601]

In sum, the concerns stated during public comment are unalloyed by your responses; aerial spraying should be eliminated until it can be shown that significant drift does not occur.
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[601] V. Breeze et al.1992 Dec. 'Use of a model and toxicity data to predict the risks to some wild plant species from drift of 4 herbicides' Ann Appl Biol:121:669-77.

Direct Rebuttals of EIS' Herbicide Chronic Toxicology Claims

PICLORAM (TORDON, GRAZON)

FEIS Claim: "Carcinogenic: Unknown"

What the Literature Says: The National Toxicology Program and World Health Organization (WHO's Int'l Agency for Research on Cancer, IARC) cancer assays are both regarded as the gold standard of cancer tests. Both of these independent tests found liver tumors in picloram exposed test animals, with IARC additionally finding thyroid tumors.[602] NTP concluded carcinogenicity evidence was equivocal; IARC concluded that picloram shows limited evidence of carcinogenicity and is currently unclassifiable. However, picloram's manufacture result in hexachlorobenzene (HCB) contamination; HCB is a probable human carcinogen according to EPA's Office of Pesticide Program's 1997 list of chemicals evaluated for carcinogenic potential, which estimates that HCB in picloram alone accounts for 70% of EPA's allowable risk for HCB exposure.[603] The exposure to HCB of ground applicators of picloram to exceed EPA's acceptable cancer risk level by ten-fold.[604] HCB causes cancer in test animals at very low doses: 0.02 ppb in drinking water is calculated to cause cancer in one-in-a million animals.

FEIS Claim: "Teratogenic: No Effects"

What the Literature Says: Picloram caused umbilical hernias at all dose levels and multiple skeletal malformations at both high and low doses,[605] while male rats suffered atrophied testicles.[606] Picloram plus 2,4-D ('Tordon 202c' brand) is a very potent teratogen when fed to parent test animals--even to the father alone.[607]

FEIS Claim: "Reproductive: No Effects"

What the Literature Says: A re-review of National Cancer Institute testicular slides of picloram exposed rats and mice determined that many of the animals had testicular atrophy,[608] after initially finding no atrophy; a result that the manufacturer Dow disputed. Dow did find increased miscarriages at picloram the higher test dose/s,[609] and the State of California found increased embryo loss for the potassium salt formulation of the picloram molecule.[610] Picloram plus 2,4-D ('Tordon 202c' brand) is a very potent reproductive toxicant when fed to parent test animals--even to the father alone.[611] The same two a.i. sold as the 4Tordon75D formulation are severely toxic to test animal testicles.[612]

FEIS Claim: "Mutagenic: Unlikely"

What the Literature Says: The National Toxicology Program found that chromosome aberrations and sister chromatid exchanges (SCEs) increased in frequency in hamster ovary cells exposed to picloram.[613] Picloram twice again tested positive for mutagenicity in tests.[614]

It is worth noting that EPA's official non-cancer safe dose estimate (RfD) for picloram is 0.07 mg/kg of body weight per

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[602] NTP 1997 Report TR-23, 1978; also the IARC picloram monograph.
[603] EPA/OPP 1996 Picloram RED.
[604] EPA/OPP 1996 Picloram RED.
[605] California Dpt. of Food & Agriculture Medical Toxicology Branch 1988 'Summary of Toxicological Data, Picloram' Sacramento CA.
[606] EPA/Office of Drinking Water (ODW) 1988 'Picloram Health Advisory' Wash. DC.
[607] Blakley et al. 1989 (3 papers).
[608] M. Reuber 1981 'Carcinogenicity of Picloram' J. Toxicol. & Env. Health:7:2:207-222.
[609] EPA 1995 (Picloram RED).
[610] Calif. DF&A 1988.
[611] P. Blakley et al. 1989.
[612] Oakes et al. 2002 (a series of papers).
[613] Calif. DF&A 1988.
[614] Muhammed et al. 1993 Mutat. Res.:426:2:193-199; and Verikat et al. 1995 Environ. Mol. Mutagen.:25:1:67-76.

day, found in EPA's Integrated Risk Information System (IRIS) database. But the main Q-RA that the USFS is relying on uses a picloram RfD almost 3 times higher (less safe), 0.2 mg/kg b.w./day (which happens to be the same as Dow's, the manufacturer of picloram. Nevertheless, there are a few modeled exposures in that Q-RA (which models exposures of pesticide applicators, the general human population, aquatic and terrestrial organism) that exceed the more lenient RfD. How many more would exceed it if they had used the stricter RfD?

TRICLOPYR (GARLON, TURFLON)

FEIS Claim: "Carcinogenic: No Effects"

What the Literature Says: Triclopyr's carcinogenicity has been studied in rats and mice. In both species, feeding of triclopyr significantly increased the frequency of breast cancer.[615]

FEIS Claim: "Teratogenic: No Effects"

What the Literature Says: Triclopyr caused kidney defects in dogs at 1/10th the dose that the manufacturer Dow found other effects, but Dow persuaded EPA that dog's excretion of triclopyr is slower than humans'; so EPA called this a "non-toxic" response and approved triclopyr for use at the necessary application rates.[616]

FEIS Claim: "Reproductive: No Effects"

What the Literature Says: Triclopyr caused reproductive effects in tests on rabbits and mice species.[617] Its major metabolite, TCP, disrupts the development of the nervous system that occurs in fetuses, infants, and children. TCP inhibits the growth of nerve cells at just 0.2 ppm, and it accumulates in the brains of primates. 2 ppm of TCP inhibits mitochondrial function, and TCP has various other chronic effects and is mobile and persistent, as this family of herbicides generally are.[618]

FEIS Claim: "Mutagenic: No Effects"

What the Literature Says: In a study of female rats mated with males who had been dosed with triclopyr, the frequency of embryo loss increased at the middle and high dose (7 and 70 mg/kg).[619]

CLOPYRALID (LONTREL-T, TRANSLINE, STINGER, CONFRONT)

FEIS Claim: "Carcinogenic: No Effects"

What the Literature Says: EPA has registered this herbicide (i.e. determined there is no unreasonable risk) without even evaluating carcinogenicity; and as of 1998 there was no public information available regarding carcinogenicity.[620] Because many cancers involve physical damage to DNA--i.e. mutagenicity--it seems likely that this carcinogenicity data gap is related to clopyralid's mutagenicity data gap, below.

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[615] EPA/OPP 1996. 'Carcinogenicity Review for Triclopyr' Wash. DC.

[616] EPA/OPP 1998 'RED, Triclopyr' Wash. DC.

[617] EPA/OPP 1998 (triclopyr RED).

[618] Hunter et al. 1999 'Gestational exposure to chlorpyrifos: Comparative distribution of trichloropyrridinol in the fetus & the dam' Toxicol. Appl. Pharmacol. 158:16-23. (TCP is also a common metabolite of insecticide chlopyrifos).

[619] EPA/OPP 1998 (triclopyr RED).

[620] EPA/OPP 1998 'List of chemicals evaluated for carcinogenic potential', a June 10 internal memo. See also personal communication to Caroline Cox (NCAP) from Rick Whitting, EPA/OPP on Nov. 19 1998, described in Cox Winter 2000 J. Pesticide Reform:20:4:12-19.

FEIS Claim: "Teratogenic: No Effects"

What the Literature Says: hydrocephaly and "[multiple] skeletal abnormalities were evident at all dose levels tested."[621] A single ingested dose of clopyralid after 1 hr. caused certain rat gastrointestinal cells to intensively secrete hormones. The cells' cytoplasm and secretory granules were covered with vacuoles.[622]

FEIS Claim: "Reproductive: No Effects"

What the Literature Says: EPA's reviewer called clopyralid's reproductive effects "substantial" and occurring in the mother rabbit at all dose levels.[623]

FEIS Claim: "Mutagenicity: No Effects"

What the Literature Says: The major medicine & toxicology databases were searched by plaintiffs for published independent literature on clopyralid mutagenicity; and none was found. Plaintiff's FOIA-requested EPA's clopyralid mutagenicity data. A 1982 EPA internal memo on the registration of a clopyralid product indicates that three Dow (the manufacturer) mutagenicity studies found clopyralid to be non-mutagenic. However, a 1987 EPA memo summarizing a clopyralid mutagenicity study states that that study is of unacceptable quality (then, a 1991 EPA summary memo states why the additional data that EPA required makes this study (finding no mutagenicity) valid.[624] Overall, it is notable that clopyralid has a complete data gap for mutagenicity in the published scientific literature, whether independently peer-reviewed or not--such widely used chemicals are almost always have mutagenicity tests results published. All that is known is that the manufacturer claims that it is not mutagenic (there is no indication that EPA audited or investigating these studies), and that other chemicals in the clopyralid family--picloram and triclopyr--are.

HEXAZINONE (VELPAR, PRONONE)

FEIS Claims: "Carcinogenic: Unlikely"

What the Literature Says: Evidence of carcinogenicity is equivocal.[625] Hexazinone tested negative for carcinogenicity except in mice at the 300 mg/kg b.w./day dose.[626]

FEIS Claims: "Teratogenic: Unlikely"

What the Literature Says: Maternal dosing of rats above 400 mg/kg b.w./d caused birth defects.[627] Some developmental effects also occurred at higher dosing levels.[628]

FEIS Claims: "Reproductive: Unlikely"

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621 EPA/OPP 1991 '..(clopyralid): Review of Rabbit Teratology Study Submitted by the Registrant', internal memo from T. McMahon, Mar. 20.

622 V. Iaglov & I. Ptashekas 1989 'The Reaction of Endocrine Cells of the Gastrointenstinal Tract in Response to exposure to 3,6-dichloropicolinic acid' Biull. Eksp. Biol. Med. 107:6:758-61.

623 EPA/OPP 1991 (clopyralid review memo). **Also**: C. Cox Winter 1998 Editorial J. Pesticide Reform:18:4:inside front cover.

624 Personal communication 24 Dec. 2002, EPA/OPPTS FOIA response to Tony Tweedale, Missoula MT.

625 EPA/OPP 1994 'RED Hexazinone Facts Summary'.

626 Weed Society of America (WSA)1994 Herbicide Handbook 7th Ed.

627 WSA 1994.

628 EPA/OPP 1994 (Hexazinone RED Summary).

What the Literature Says: Reproductive effects occurred at the mid and high dose levels--hardly “unlikely”.[629]

FEIS Claims: "Mutagenic: No Effects"

What the Literature Says: The RED Facts summary says one mutagenicity test was positive. However some live animal mutagenicity tests were “inconclusive”.[630]

It is worth noting that hexazinone’s RfD (“safe” dose) is 0.033 mg/kg b.w./day.[631] The NOEL the RfD is based on was 10 mg/kg b.w./d, significantly lower than the doses above causing teratogenicity and cancer. It was noted that the NOEL study was not an acceptable chronic exposure study, so that the real NOEL is unknown . Therefore an extra 3-fold “safety” factor was added to the RfD (total 300-fold safety factors), as though this data gap were actually a known quantity. In any case, the hexazinone RED Facts summary cites a different NOEL, 5 mg/kg b.w./day--half that other “NOEL”. Using that true (lowest known) NOEL, but the same safety factors (because this NOEL was derived from just a 1 yr dosing regime), hexazinone’s RfD should be 0.0167 mg/kg b.w./day; i.e. all risk estimates would double (assuming, as always, that a lower NOEL is not discovered).

METSULFURON METHYL, SULFOMETURON METHYL (OUST) AND CHLORSULFURON (GLEAN, TELAR)

FEIS Claims: All three sulfonureas (SUs) "No Effect" in all four categories (except sulfometuron methyl (Oust): "Reproductive: Unlikely").

What the Literature Says: Sulfometuron methyl disrupts reproduction in several ways. Both rats and dogs had various testicular abnormalities, including testicular atrophy.[632] Sulfometuron methyl also caused smaller litters in both rats and rabbits.[633] Similar effects occurred in the test of a SU drug).[634]

As to carcinogenicity and mutagenicity: although DuPont’s mutagenicity tests on sulfometuron methyl were negative, sulfometuron methyl metabolizes to saccharin,[635] a mutagen and potent carcinogen (though controversial as to carcinogenicity in humans). One of the known ingredients in sulfometuron methyl (and likely in other SU herbicide formulations), therefore untested during registration, is polyvinyl pyrrolidone, which causes various cancers (mostly sarcomas) in mice, rats and rabbits when tested by the International Agency for Research on Cancer.[636] The blood of dogs chronically dosed with sulfometuron methyl in feed is severely affected, both red and white (immune) cells. Anemia was very evident. Anemia is strongly associated with leukemia, and cancer of the immune system. The liver and kidneys were also affected in these studies.[637]

SUs, such as these three herbicides, stimulate human insulin secretion, which must be kept in balance to avoid disease. Diabetes is a complex disease, associated inter-alia with heart disease. SU herbicides (which also are used as diabetes drugs) affect the critical sodium pump of myocardial cells and have been associated with cardiovascular mortality in humans.[638] In the many people who take sulfoamide antibiotics, taking an SU drug for diabetes will cause hypoclycenia, as sulfoamide antibiotics inhibit the metabolism of the SU drug.[639] SUs can affect thyroid hormone production and

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[629] EPA/OPP 1994 (Hexazinone RED Summary).

[630] Weed Soc. 1994.

[631] USFS & Bonneville Power Admin. 1992 ‘Risk Assessment for Herbicide Use...’.

[632] EPA/OPP 1 Dec. 1983 ‘E. I. DuPont Oust Weed Killer’, internal memo of A. Arce to R. Taylor, Wash. D.C. Also EPA/OPP 23 Feb. 1993 ‘Sulfometuron Methyl-Evaluation of two-generation,...’, internal memo of R. Fricke to L. DeLuise, Wash. D.C.

[633] EPA/OPP 23 Feb. 1993 Sulfometuron methyl memo; also EPA/OPP 26 Oct. 1981 ‘Registration of new pesticide: Oust Weed Killer’, internal memo of W. Dykstra to R. Taylor, Wash. D.C.

[634] Seyler et al. 1992 Reprod. Toxicol.:6:447-452.

[635] EPA/OPP 6 Sept. 1991 ‘Pesticide environmental fate one-line summary: sulfometuron methyl’, Wash. D.C.

[636] IARC 1999 ‘N-Vinyl-2-pyrrolidone and polyvinyl pyrrolidone’ IARC Monographs:71:1181.

[637] EPA/OPP 1983 Memo from A. Arce to R. Taylor of E. I. DuPont, 13 Oct. (document ID#353-UNR).

[638] Dennis Kim & Steven Edelmana 22 Feb. 2001. An answer by MD’s to a Q&A on Medscape's web site (http://www.medscape.com).

[639] D. Juurlink et al. (2 Apr. 2003) ‘Drug-Drug Interactions Among Elderly..’ JAMA:289:13:1652-8 (refer’s 24-30).

balance.[640]

The imidazolinone and SU families of herbicides, whose mechanism of action is acetolactase synthase (ALS) enzyme inhibition in plants, are applied at remarkable low rates. Broad spectrum SU's such as Oust put vast tracts of native vegetation at tremendous risk.[641] Following unwanted crop reductions in regions where SUs were being used, EPA did controlled field tests of chlorsulfuron. Fruit yields were reduced at levels as low as a thousand times less than the recommended application rate. At less than one hundredth the application rate, various crops suffered yield loss.[642] But EPA's phytotoxicity tests when registering pesticides does not consider SU's mode of action which attacks plant reproduction (it doesn't consider 80% of a plants life cycle).[643] Benlate, a fungicide apparently contaminated with SU's made at the same DuPont plant, has generated a flood of lawsuits for unexpected damage to crops.[644] This would be consistent with the extraordinarily low rates this herbicide works at, for either target or non-target vegetation. Despite the extreme potency of SU and imidazolinones family of herbicides, weeds are growing resistant to them, as is inevitable (see our discussion of resistance)--73 documented species have shown resistance, so far.[645]

IMAZAPYR (ARSENAL, CHOPPER, ASSAULT...etc.) AND IMAZAPIC (PLATEAU, CADRE, ...)

FEIS Claims: "Carcinogenic: Unknown"

What the Literature Says: Though judged that there is evidence imazapyr is *not* a carcinogen, those tests did show increased brain, thyroid and adrenal tumors in the test animals. Yet EPA found that except for the brain tumors, the increases were not greater those found in other tests.[646] This appears to leave open the possibility that the increase was statistically significant and that imazapyr should have been classified as a possible or actual carcinogen, especially considering the registrant controlled the test. As to the brain tumors, EPA allowed Amer. Cyanamid to re-analyze the pathological slides, after which EPA agreed with a finding that there was evidence of one additional brain tumor in both the dosed and the control animals, which caused the overall increase in brain tumors to move below the level of statistical significance.[647] Even short of fraud, it is unusual for an accepted cancer assay result to have its raw results changed on re-analysis.

Similarly with imazapic, it was classified by EPA as Group E--evidence of non-carcinogenicty--even though the pesticide registration test showed more thyroid tumors and cancers than the unexposed rats.[648] The only other ingredient in imazapic formulations which EPA has disclosed so far[649] is crystaline silica, a potent known human carcinogen when inhaled[650] (as can happen after an application dries).

FEIS Claim: "Reproductive: Unknown; Teratogenic & Mutagenic: No Effects""

As of 1996 there was no public information available on whether reproductive risks were unreasonable enough not to register imazapyr (first registered in 1984, reviewed in 1992). The chronic effects test literature for imazapyr does include lung edema, kidney cysts, blood cell malformation, brain congestion and brain and thyroid cancers and adrenal

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640 R. Guazelli et al. 1968 Acta Diabetol. Latina:5:614-623; and: J. Hershman et al. 1968 J. Clinical Endocrin.:28:1605-1610.

641 Short & Colburn 1999 _Toxicol. & Industrial Health_:15:240-275 (summarizing all this data).

642 J. Fletcher, T. Pfleeger & H. Ratsch 1993 'Potential environmental risks with the new sulfonurea herbicides' Environ. Sci. Technol. 27:2250-2252. **Also** J. Fletcher et al 1995 Physiol. Plantarum:94:261-7 **and** J. Fletcher 1996 Env Toxicol & Chem:15:1189-96.

643 Journal of Pesticide Reform Fall '96 16:3:10-11.

644 Multinational Monitor Jul./Aug. '93, p.4.

645 I. Heap 2002 (i.e. the Weed Science Soc. Amer., http://www.weedscience.org).

646 EPA/OPP 1991 'Peer Review of Imazapyr' Oct. 2. memo from W. Dykstra.

647 C. Cox 1996 'Imazapyr Herbicide Fact Sheet', J. Pesticide Reform 16:3:16-17.

648 EPA/OPP/HED 2001 'Imazapic: Report of the Haz ard Identification Review Cmtee Memo from W. Dykstra to W. Donovan'. Wash, DC May 3.

649 C. Cox 2003 'Imazapic Factsheet' J Pesticide Reform:23:3:10-14 (see p. 10-11, 'Inerts').

650 Int'l Agency for Research on Cancer (IARC) 1997 'Monograph 68:41; avail. at: http://cie.iarc.fr/htdocs/monogrpahs/vol68/silica.ht

tumors.[651]

Similarly for imazpic the registration test for reproductive/developmental effects showed increasing rate of undeveloped ribs in rabbits,[652] but EPA says the same effect was observed in unexposed test animals from other tests in the same lab--ignoring that the control rabbits in this test, i.e. under the exact same conditions as the exposed rabbits--did not develop this defect, much less in a dose/response manner. In the registration general chronic effects test imazapic caused muscle degeneration at all dose levels, anemia at the mid & hi dose levels, liver enlagement at the mid & hi-doses (with enzymes that mark liver disease at the hi-dose), and elevated cholesterol at the mid-dose level only.[653]

In addition to acute toxicity to non-target plants, a variety of other impacts have been reported.[654] These include hazards to endangered species, increased susceptibility to disease, and disruption of nutrient cycling in soil. Separate NOELs exists of 50 and 150-175 mg/kg bw/d.[655] Obviously the latter is not the NOEL; the former is, unless there is an even lower NOEL. Therefore imazapyr's non-cancer health risks were underestimated at least 3-fold. It's interesting to note that a herbicide with an all but identical structure, imazapic, has a RfD of just 0.05 mg/kg b.w./day, according its manufacturer BASF.[656] Thus its NOEL is no more than 5 mg/kg bw/d (i.e. taking away the RfD's minimum 100-fold safety factor). This is some 30 times lower than the NOEL of imazapyr, structurally similar.

This imidazolinone family herbicide has a mechanism of action on plants the same as the sulfonurea family (inhibition of acetolactate synthase enzyme, ALS, essential to production of three amino acids that mammals rely on plants for), thus it harms native vegetation at similarly minute doses as the SUs (e.g. 1/100th an ounce/acre or 1/18th to 1/135th the recommended application rate, already extrordinarily low).[657] In the temporal and geographic vicinity of organophosphate insecticides, these herbicides are even more (synergistically) potent.[658]

DICAMBA (BANVEL, part of TRIMEC, ...)

FEIS Claim: "Carcinogenicity: No Effects"

What the Literature Says: Dicamba is akin to the 2,4,-D molecule . As with the similar molecule 2,4-D, exposure to dicamba is strongly associated with Non-Hodgkin's Leukemia (NHL)--and farmer's use of dicamba is associated with a doubling of their NHL,[659] as are the dioxin contaminants that dicamba's manufacture creates. The predominant dioxin found in dicamba is 2,7-DCDD, which causes several cancers in lab animals and numerous other defects.[660] The amine salt version of dicamba is contaminated with potent oxidative-damage carcinogens such as dimethyl nitrosamine.[661]

FEIS Claim: "Teratogenic: No Effects"

What the Literature Says: Several birth defects are caused by dicamba (and/or its potent contaminants) at low doses.[662]

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[651] EPA/OPP 1989, '90 & '91 (2 Data Evaluation Reports & a Peer Review, all for imazapyr, by EPA's W. Dykstra).

[652] EPA/OPP/HED 2001.

[653] EPA/OPP/HED 2001.

[654] Cox 1996 (Imazapyr Fact Sheet).

[655] Weed Society of America 1994 'Herbicide Handbook' 7th Ed.

[656] USFS 2001 'LNF BGWR&BAWM FEIS'; see table IV-16, p. IV-50 & 51.

[657] J. Burns et al. 1999 'ALS Inhibitors Increase Eth[]ene Production & Cause Fruit Drop in Citrus' Hort Science:34:908-10 **and** EPA/OPPT/EEB 1995 'Env. Risk assmnt. for the Use of Imidilazolinonne Type herbicide CADRE on Peanuts. Memo from A. Maciorowski to R. Taylor, Registration Div.' Wash. DC 25 Aug. **and** S. Ranayke & D. Shaw 1992 'Effects of Harvest-Aid Herbicides on Sicklepod...' Weed Technol.:6:985-9; **also** J. Fletcher 1993 (using SUs).

[658] R. Hartzler et al. 2000; **also see** the Plateau DG & Cadre DG labels by BASF Corp. 2000 & 2002, which warn of crop loss (see http://cdms.net).

[659] K. Cantor 1992 Cancer Res. 52:2447-2455.

[660] J. Huff et al. 1991 Env. Health Perspectives 93:247-270.

[661] ?, Pure & Applied Chem. 52:499-526 1980 Int'l Union of P&AC (IUPAC).

[662] EPA/ODW 1968 'Dicamba Health Advisory' Wash. DC. Also Federal Registers 48:52:11,113-4 and 11,119-20)

FEIS Claim: "Reproductive: Unlikely"

What the Literature Says: Spontaneous abortions/fetal resorbtions occur in rabbits at fairly low dose, above 3 mg/kg body weight/day.[663] Mallard egg development is stunted.[664] The contaminant 2,7-DCDD (a dioxin) is also a reproductive toxicant, among its numerous potent effects.[665]

FEIS Claim: "Mutagenicity: No Effects"

What the Literature Says: Dicamba significantly increases DNA unwinding, unscheduled DNA synthesis and causes sister chromatid exchanges.[666] Four earlier studies also show it is mutagenic, including in applicators.[667]

GLYPHOSATE (ROUNDUP)

FEIS Claim: "Carcinogenicity: No Effects"

What the Literature Says: After a dispute with the manufacturer Monsanto over interpreting the Monsanto's glyphosate carcinogenicity studies, which showed at least some evidence of various cancers, EPA's conditional conclusion in the 1980's was that glyphosate shows some evidence of non-carcinogenicity, pending further study.[668] One EPA OPP staff person termed Monsanto's data suspicious, given the need to protect health .[669] Since that suspicious dispute, glyphosate exposure has since been associated with non-Hodgkin's lymphoma, NHL, in a small non-significant case-control group.[670] There exists an un-cited reference to a 1999 study of Swedish farmers, where glyphosate use correlates with a more than doubled rate of NHL. A recent Swedish study of the rare hairy cell leukemia (HCL, a form of NHL), found that people who were occupationally exposed to glyphosate formulations had a threefold higher risk of HCL, and a similar risk for NHL.[671] Glyphosate is again associated with significantly elevated risk of the rare hairy cell leukemia.[672] A 2003 study confirmed the association of glyphosate exposure with increased incidence of non-Hodgkin's lymphoma.[673]Even an industry study found 'somewhat elevated' rates of various cancers in mice given glyphosate.[674] Roundup is a potent steroid hormone disrupter.[675] EPA warned the Drug Enforcement Agency in 1985 that glyphosate increases male animal kidney tumors, in a dose-dependent manner.[676] Overall, it's interesting to note that immune suppresion is strongly associated with cancers of the immune such as NHL & HCL, and glyphosate mutagenicity studies also support this association.[677]

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[663] EPA/ODW 1988.

[664] Hoffman et al. 1984 Arch. Env. Contam. & Toxicol.:13:15-27.

[665] Khera & Ruddick 1973, Adv. Chem. Ser.:120:70-84.

[666] P. Perocco et al. 1990 Env. Mol. Mutag.15:131-135.

[667] Plewa et al. 1984; Ma 1984; Yoder et al 1973 **all in** Mut. Res. (138:233-245; 138:157-167; 21:325-330); **and** Puaztal 1986 Acta Botany Hung.:32:163-168.

[668] EPA/OPP 1991 'Second Peer Review of Glyphosate', internal Memo from W. Dykstra & G. Ghali, Oct. 30. Also 3 preceding OPP documents on this issue, all cited in C. Cox 1998 'Glyphosate Factsheet' J. Pesticide Reform:18:3:3-16.

[669] EPA/OPP 1985 'Use of Historical Data in determining the Weight-of-the-Evidence From Kidney Tumor Incidence in the Glyphosate...and Some Remarks on False Positives', internal Memo from Herbert Lacayo 26 Feb.

[670] Hardell & Eriksson 1999.

[671] M. Nordstrom, L. Hardell et al. 1998 'Occupational exposures, animal exposure and smoking as risk factors for hairy cell leukemia evaluated in a case-control study'. British Journal of Cancer 77:11:2048-2052 (for both studies).

[672] L Hardell et al. 2002 'Esposure to pesticides as a risk factor for non-Hodgkin's lymphoma and hairy cell leukemia: pooled analysis of two Swedish case-control studies' Leuk. Lymphoma:43:1043-1049.

[673] A DeRoos et.al. 2003 'Integrative assessment of multiple pesticides as risk factors for non-Hodgkin's lymphoma among men' Occup. Environ. Med.:60:11-17.

[674] K. Pavkov & J. Turnier1986 '2-Year Chronic Toxicity & Oncogenicity Dietery Study With SC-0224 in Mice'. Report # T-11813, Farmington: Stauffer Chemical Co.

[675] Walsh et al 2000 Env. Health Perspectives:108:769-776.

[676] Pesticide & Toxic Chemical News 14 Aug. '85, p.8.

[677] Hardell & Eriksson 1999.

FEIS Claim: "Teratogenic: No Effects"

What the Literature Says: Minnesota farm families that used Roundup regularly had statistically significant increases in birth defects (and a 3-fold increase in neuro-developmental disorders). That study also found a tentative association with attention deficit/hyperactivity disorder (ADD/ADHD)."[678] A study of pregnant rats given glyphosate in their drinking water showed that this exposure caused changes in the activity of three enzymes in their fetuses--enzymes related to energy production were affected in the liver, heart, and brain.[679] Preconception glyphosate (among other herbicide exposures) was associated with a 20-40% relative increase in adverse birth outcomes; and glyphosate specifically was associated with late abortion, regardless of when exposure occurred.[680]

FEIS Claim: "Reproductive: Unlikely"

What the Literature Says: In rats, glyphosate reduced sperm counts at the two highest doses tested. In male rabbits, glyphosate at doses of 1/10 and 1/100 of the lethal dose increased the frequency of abnormal and dead sperm.[681] Human father's use of glyphosate correlates with increased miscarriages and premature births in farm families.[682] Women's exposure to glyphosate among other herbicides and insecticides before conception is associated with a 20-40% increased risk of spontaneous abortion after conception, with older women's apparent risk being much higher for at least some of the pesticides.[683] A case report of frequent menstruation from a student using a track where glyphosate was sprayed.[684] Contrary to the label's claim of safety to pets if used as directed, a case report of dog miscarriage from a man's glyphosate-sprayed yard.[685] In a study of female rabbits given glyphosate orally during pregnancies, glyphosate caused a "slight" decrease in fetal weight in all three treated groups.[686]

Although an ecologic effect, Roundup formulation had the biggest effect on tadpole mortality of four pesticides tested, wiping out two species and nearly a third; in a controlled experiment that closely simulated natural conditions (using 25 aquatic plus algal species) and using the maximun allowed application rates to simulate direct overspray; also, this effect was direct--not due to toxicity to the tadpoles' algal food.[687] Following-up, Relyea found that Roundup formulation killed frogs on land (79% in a day) as well as tadpoles in water (98% within 3 weeks), across three families of species, in a worst-case (i.e. direct overspray) yet still legal application rate. These lethalities occurred at Roundup levels 10-100X lower than some earlier amphibian studies (judging from the journal and dates of those studies were in, they may've been the studies performed for registration).[688] A companion follow-up study found that just a third of the legal application rate in a direct overspray of a pond with Roundup (resulting in 1.3 mg/L) reduced tadpoles by 40%, and disrupted the food chain.[689]

In short, though Monsanto endlessly bleats that Roundup's POEA surfactant (detergent) is safe, it has yet to publish any such result in an objective journal. That's most likely because Monsanto is lying, given that at least six objective experiments have found that is deadly to tadpoles and other developing amphibians. At least two (the first and last cited)used real-world doses, e.g. the latter used the estimated concentration at 42 days (POEA's estimated half-life) after

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678 V. Garry et al June 2002 'Birth defects, season of conception, and sex of children born to pesticide applicators living in the Red River Valley of Minnesota, USA' Env Health Perspectives:110(Suppl. 3):441-9.

679 Daruich et al. 2001 'Effect of herbicide glyphosate on enzymatic activity in pregnant rats and their fetuses' Environ. Res./Sect. A 85:226-231.

680 Arbuckle et al. 2001.

681 M. I. Yousef et al. 1995. 'Toxic effects of carbofuron and glyphosate on semen characteristics in rabbits' J. Env. Science Health/sec. B 30:4:513-534.

682 D. A. Savitz, 1997. American Journal of Epidemiology:146:1025-103.

683 Arbuckle et al. 2001.

684 Barnard & Heauser in NCAA Sports Sciences Education Newsletter Vol. 2 Fall 1995.

685 J. of Pesticide Reform Fall '98, letters.

686 EPA/Off. of Toxic Substances 1980 'Glyphosate submission of rat teratology, rabbit teratology' Reg. #524-308.

687 R. Relyea Apr. 2005 'The Impact of Insecticides and Herbicides on the Biodiversity & Productivity of Aquatic Communities' Ecol. Applicattons:15:2:618-27.

688 R A Relyea Aug. 2005 'The Lethal Impact of Roundup On Aquatic & Terrestrial Amphibians' Ecological Applications:15(4): 1118-24.

689 R A Relyea Aug. 2005 'Pesticides & Amphibians: the importance of community context' Ecological Applications:15(4): 1125-34.

a label-rate Roundup application.[690] Also, aerial applications have dosed small wetlands with up to 1.9 mg of acid equivalents per liter of Roundup, despite the herbicide's label "restrictions".[691] That's a concentration higher than that which caused tadpole mortality & abnormalities in that last of the seven studies![692]

FEIS Claim: "Mutagenic: No Effects"

What the Literature Says: Even if it is falsely (as shown here) assumed that glyphosate is not mutagenic, aa study showed that it was when cells are undergoing oxidative damage stress.[693] Mice injected with glyphosate and Roundup witnessed increased frequency of chromosome damage and DNA damage increased in bone marrow, liver, and kidney.[694] In fruit flies, Roundup and Pondmaster both increased the frequency of sex-linked, recessive lethal DNA mutations, showing that the formulation is very mutagenic.[695]The 1997 study, also testing the a.i. vs. the formulation, found that human lymphocytes showed an increase in the frequency of sister chromatid exchanges following exposure to glyphosate in all but the lowest doses.[696] Glyphosate caused sister-chromatid exchanges in human lymphoid cells[697] Even in studies by the manufacturer, it caused a variety of chromosone aberations and gene mutations in mice lymphoid cells,[698] supporting the above correlations with immune cancers. An unidentified ingredient in the ubiquitous glyphosate formulation 'Roundup' (not the glyphosate itself) caused more DNA adducts in mice as the dose increased.[699] A team in France has published at least four findings of glyphosate formulation's toxicity to cell replication and gene transcription, including synergy with formulation ingredients on cell division (J. Marc et al 2005).[700]

Ironically, Monsanto, the exclusive manufacturer, markets glyphosate by emphasizing it is allegedly far safer than other herbicides, e.g.: "God's herbicide [because it's] -- safer than salt". The EPA and the NY Attorney General took enforcement action for false and illegal claims of safety under FIFRA and FTC marketing rules, after such violations were brought to their attention.[701] As NCAP notes, glyphosate has been shown to be toxic in every standard category of toxicology testing.[702]

In addition to glyphosate/Roundup's toxicity, Denmark recently banned most uses of it after their Denmark & Greenland Geological Research Institution found it not degraded by soil microbes--as long claimed by Monsanto and widely believed--but rather they found it in shallow groundwater at 0.54 ug/L concentration.[703] Monsanto claims that its detection in groundwater at one meter below the surface does not show it reaches drinking water. Also contradicting the general supposition by herbicide proponents that these pesticides are not persistent are studies showing how herbicides such as glypphosate, 2,4-D and picloram/triclopyr/clopyralid harm fruits and vegetables composted with mulch that had

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690 Relyea 2005 **and:** Lancet 1988, 1, 299; Arch. Environ. Contam. Toxicol. 1999, 36, 193–199; Environ. Pollut. 2001, 114, 195–205; Chemosphere 2003, 52, 1189–1197; Environ. Toxicol. Chem. 2004, 23, 1928-38.

691 ? 2004 '?' Environ. Toxicol. Chem.:23:843–849.

692 R. Renner 2005.

693 A. Lueken et al. 2004

694 C. Bolognesi et al. 1997 'Genotoxic activity of glyphosate and its technical formulation Roundup' J. Agricultural Food Chemicals 45:1957-1962.

695 P. Kale. et al. 1995. 'Mutagenicity testing of nine herbicides and pesticides currently used in agriculture' Environ. Mol. Mutagen. 25:148-153. Also Peluso et al. 1998 Environ. Mol. Mutagen. 31:55-59.

696 C. Bolognesi. et al. 1997.

697 N. Vigfusson & E. Vyse 1980 'The Effect of the Pesticides...and Roundup on Sister-Chromatid Exchanges in...' Mutag. Res.:79:53-57.

698 J. Majeska & D Matheson Reports #T-10848, #T-11018 on compound R-50224 (1982), **and** reports #T-12661, #T-12662 (the chromosone aberations result) on compound SC-0224 (1985); both by Farmington: Stauffer Chemical Co.

699 Peluso M 1998 '32P-postlabeling detection of DNA adducts in mice treated with the herbicide Roundup' Environ Mol Mutagen.:31:1:55-9.

700 'J. Marc et al. 15 Feb. 2005 'A glyphosate-based pesticide impinges on transcription' Toxicol Appl Pharmacol.:203(1):1-8. J. Marc Dec. 2004 'Formulated glyphosate activates the DNA-response checkpoint of the cell cycle leading to the prevention of G2/M transition' Toxicol Sci.:82(2):436-42. J. Marc Apr. 2004 'Glyphosate-based pesticides affect cell cycle regulation' Biol Cell:96(3):245-9. J. Marc et al. 2005.

701 NCAMP 1997 Technical Rpt. 12:2.

702 C. Cox Fall 1998 'Glyphosate Factsheet' J. of Pesticide Reform:18:3:3-16.

703 A.L. Schmidt 10 May 2003 'Poisonous Spray on Course Towards Drinking Water' Politiken, Denmark (avail.: http:politiken.dk/VisArtikle.sasp?PageID=269614).

been treated with those herbicides.[704]

This last fact, as with the above descriptions, illustrates the Orwellian nature of the claims made about "God's pesticide" (i.e., to denote its suppose total safety). There simply is no other pesticide with such extravagant claims made about its safety; where upon independent investigation, the truth turns out opposite. Concentrated evil.

2,4-DICHLOROPHENOXY ACETIC ACID (2,4-D)

FEIS Claim: “Teratogenic: Unlikely

What the Literature Says: Both 2,4-D salts (including the amine) were teratogenic when the Ntl. Cancer Institute tested them before 1970.[705] Embryo deaths and kidney & urogenital defects resulted in 2,4-D experiments.[706] Increased spontaneous abortions resulted in a 2,4-D experiment.[707] Supernumery ribs resulted from 2,4-D dosing.[708] Tests on rats showed 2,4-D caused multiple rib malformalities and slow backbone formation at higher doses--the same category of defects as found, inter alia, in the following epidemiology studies.[709] In human populations, 2,4-D is significantly associated with spontaneous abortions in women exposed 3 months prior to conception, possibly from 2,4-D in the semen of farmers.[710] Throughout rural Minnesota, birth defects were more frequent when parents were carefully estimated to have been exposed to one of the two currently registered chlorophenoxy herbicides, usually 2,4-D.[711] Those results were just confirmed in a different population, finding that human conception during the spring herbicide spraying season in 147 high wheat-producing counties (88% of wheat acreage uses chlorophenoxy herbicides (mostly 2,4-D, some MCPA) led to five times the rate of musculoskeletal & circulatory birth defects, compared to low/non-wheat producing rural counties; while year-around conceptions in the chlorophenoxy-using counties resulted in just twice the rate of birth defects. Birth certificates, used here, are known to significantly underreport birth defect, which would affect the high-defect 2,4-D using counties more. The more the several different pesticides used in the low/non wheat-producing rural counties cause birth defects (as many pesticides do), the more the birth defect correlation found with 2,4-D would be underreported.[712] In traditional high dose (ppm) animal studies, 2,4-D caused bleeding of the abdominal cavity of rat fetuses.[713]

FEIS Claim: "Reproductive: Unlikely"

What the Literature Says: 2,4-D is found in the semen of agricultural workers.[714] Men with higher body burdens of 2,4-D had significant levels of semem abnormalities.[715] Sperm quality and quantity were severely affected in 32 farmers using 2,4-D (urinary verification);[716] corroborated by the Minnesota farmers teratogenicity study where in addition birth

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[704] R Stocker et al. Nov. 1999 'Residual Effects of Herbicide-Treated E. Crassipes Used aas a Soil Ammendment' Hydrobiologia:415:329-33; **and** see media reports of this problem caused by the picloram/triclopyr/clopyralid.

[705] Reported in Trial, Nov. 1983 p. 97; and J. Schardein ed. 1983 Chemically Induced Birth Defects 2nd Ed., New York:Marcel Dekker.

[706] D. Fofana et al. 2000 'Prenatal Developmental Effects of Pure 2,4-D Acid on the Rat' Congen.Anomal.:40:287-296.

[707] T. Arbuckle et al. 1999 'Exposure to Chlorophenoxy Herbicides and the Risk of Spontaneous Abortions' Epidemiology:10:752-760.

[708] N. Chernoff et al. 1990 'Effects of Chemically Induced Maternal Toxicity On Prenatal Development In the Rat' Teratology:42:651-658.

[709] EPA/OPP 1996 ‘2,4-D Acid: Review of Chronic Toxicity/Carcinogenicity...’ 23 May docket memo to the Special Review & Re-registration Branch. Also Chernoff et al. 1990 Teratology 42:651-658.

[710] T. Arbuckle et al 2001 'An Exploratory Analysis of the Effect of Pesticide xposure in the Risk of Spontaneous Abortion in an Ontario Farm Population' Env. Health Perspectives:109:851-857.

[711] Garry et al. 1996.

[712] Schreinemachers July 2003.

[713] ExToxNet 1996 '2,4-D Pesticide Information Profile', Extension Toxciology Network; available http://extoxnet.orst.edu/pips/24-D.htm, accessed 30 July 2004.

[714] T. Arbuckle et al. 1999 '2,4-D Acid Residues in Semen of Ontario Farmers' Reprod. Toxicol.:13:6:421-9.

[715] Swan et al. Sept. 2003.

[716] Lerda & Rizzi 1991 'Study of Reproductive Function in Persons Occupationally Exposed to 2,4-D Acid' Mut. Res. 262:47-50.

frequency was half that of non-exposed farmers, and leutenizing hormone levels were too high.[717] Semen and sperm quality & quantity were poorer in men with elevated 2,4-D burdens in rural (yet not occupational users of pesticides) mid-Missouri men, vs. the semen of men of urban Minnesota areas with low 2,4-D levels.[718] A single high dose of 2,4-D caused a 29% reduction in DNA synthesis in the testis of mice.[719] 2,4-D alters testicular Leydig cells.[720] High subchronic doses of 2,4-D caused testicular atropphy in rats.[721] Women using chlorophenoxy herbicides (and organophosphate insecticides) had significantly lower fecundity.[722] Picloram plus 2,4-D ('Tordon 202c' brand) is a very potent reproductive toxicant when fed to parent test animals--even to the father alone.[723] The same two a.i. sold as the Tordon75D formulation are severely toxic to test animal testicles.[724] A retrospective study of infertile women found they were almost 27 times(!) more likely to have mixed or applied herbicides (but not insecticides) than fertile women (and 3.3 times more likely to have used fungicides); after adjustment for confounding variables (though living on a farm, ranch or in a rural home reduced the likelihood of infertility; the strong health and fertility of agricultural residents is well known).[725]

FEIS Claim: "Cancer: Unknown"

FEIS Claim: "Mutagenicity: Unlikely"

Below we adopt a more extensive format (abstracts, and both positive & negative findings), so that we may prove to you our claim that even the above extensive rebuttals to your claims represent only a fraction of the independent studies published, that collectively show chronic toxicity when you claimed 'none' or 'unlikely'. **That is, the abstracts that follow are a complete literature search of 2,4-D's carcinogenicity and mutagenicity (performed recently by public interest groups for submittal of public comment on EPA's draft Risk Assessments for 2,4-D's re-registration), so they include findings of no toxicity. Almost every single published study has found that 2,4-D is carcinogenic and mutagenic.** 'Publication bias'--the urge to publish positive findings--explains part of this overwhelming inbalance, **but publication bias is irrelevant: one valid finding of toxicity is more significant than a hundred or more valid negative findings.** Thus, and given our experience in researching the toxicity of other chemicals, **there is no doubt that complete literature searches any of the other herbicides critiqued here will yield similar overwhelming findings of chronic toxicity.** Also, the published literature on other than these narrow four chronic effects just as overwhelmingly finds toxicity. **In fact, it has been our experience that findings of no toxicity are made by authors and journals receiving financial benefit from the industry that benefits from their negative findings--indeed, research has proved that such a correlation is prevalent in published papers**.[726]

GENERAL POINTS ABOUT EPA'S CARCINOGENCITY REVIEW

Regarding the di-ethanol-amine (DEA) form of 2,4-D, we protest strongly your intention not to allow public comment on the data that industry has promised to submit on DEA carcinogenicity. As you mentioned, cocamine DEA and possibly free DEA show some evidence of carcinogenicity according to the premier evaluators, IARC and the NTP. Please arrange for public notice and comment before finishing your evaluation of this chemical.

We fail to understand the logic of your conclusion, "Cancer Aggregate Risk" (p. 79). Since you believe that there is insufficient evidence to classify 2,4-D as a carcinogen, how on Earth can you claim that "The endpoint selected for the

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[717] Garry et al. 1996.

[718] S. Swan et al. 2003 'Geographic Differences in Semen Quality of Fertile US Males' Environmental Health Perspectives111:4:414-420. **And** Shanna Swan et al. Sept. 2003 'Semen Quality in Relation to Biomarkers of Pesticide Exposure' Env. Health Perspectives:111:12:1478-1484.

[719] J. Seiler 1979 'Phenoxyacids As Inhibitors of Testicular DNA synthesis in Male Mice' Bull. Env. Contam. & Toxicol.:21:1&2:89-92.

[720] R.C. Liu et al. 1996 'The Leydig Cell Function in Vitro' Fundam. & Applied Toxicol.:30:102-8.

[721] J. Charles et al. 1996 'Comparative Subchronic Studies on 2,4-D Acid, Amine & Ester in Rats' Fundam. & Applied Toxicol.:33:2:161-5.

[722] K. Curtis et al. 1999 'The Effect of Pesticide Exposure on Time to Pregnancy' Epidemiology 10:112-117.

[723] P. Blakley et al. 1989--3 papers.

[724] Oakes et al. 2002.

[725] A. Greenlee et al. 2003. 'Risk factors for female infertility in an agricultural region' Epidemiology:14:429-436.

[726] Swaen & Meijers 1988; Bekelman et al. 2003.

cPAD will be protective of the possible carcinogenic activity of this chemical."??

POSITIVE CANCER RESULTS/REVIEWS; EXPERIMENTAL

Adhering to EPA's cancer assessment guidelines--which require considering the weight of the evidence (epidemiology, experimental, mechanistic; and the quality of peer-review)--we conclude that 2,4-D is clearly at least a Class C carcinogen. Please justify your Class D (insufficient evidence) classification, including why you have not considered the quality the evidence (as measured by the proxy of the quality of peer review).

The International Agency for Research on Cancer--one of the two 'gold standards' in the world that does carcinogen assessments--has classified chlorophenoxy herbicides as possible human carcinogens since 1987 (International Agency for Research on Cancer 1987 'Chlorophenoxy Herbicides' IARC Monographs:(Suppl 7):156; http://www-cie.iarc.fr/htdocs/monographs/suppl7/chlorophenoxyherbicides.html ,accessed Jan. 2004). We ask that you explicitly justify your 'D' classification in light of IARC's expertise in carcinogenicity and in the face of the overwhelming weight of the evidence in the published, independent peer-review literature; summarized below.

An expert panel--conservative, because it was balanced between independent and industry scientists-- concluded that the weight of the evidence is that 2,4-D is clearly a potential human carcinogen (i.e. EPA Class C):

> Environ Health Perspect. 1991 Dec;96:213-22. Weight of the evidence on the human carcinogenicity of 2,4-D.
> Ibrahim MA, Bond GG, Burke TA, Cole P, Dost FN, Enterline PE, Gough M, Greenberg RS, Halperin WE, McConnell E, et al. Center for Risk Analysis, Harvard School of Public Health, Boston, MA 02115.
> The phenoxy herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) is widely used to control the growth of weeds and broadleaf plants. We convened a panel of 13 scientists to weigh the evidence on the human carcinogenicity of 2,4-D. The panel based its findings on a review of the toxicological and epidemiological literature on 2,4-D and related phenoxy herbicides. The toxicological data do not provide a strong basis for predicting that 2,4-D is a human carcinogen. Although a cause-effect relationship is far from being established, the epidemiological evidence for an association between exposure to 2,4-D and non-Hodgkin's lymphoma is suggestive and requires further investigation. There is little evidence of an association between use of 2,4-D and soft-tissue sarcoma or Hodgkin's disease, and no evidence of an association between 2,4-D use and any other form of cancer. Scientists on the panel were asked to categorize 2,4-D as a "known," "probable," "possible," or "unlikely" carcinogen or as a noncarcinogen in humans. The predominant opinion among the panel members was that the weight of the evidence indicates that it is possible that exposure to 2,4-D can cause cancer in humans, although not all of the panelists believed the possibility was equally likely: one thought the possibility was strong, leaning toward probable, and five thought the possibility was remote, leaning toward unlikely. Two panelists believed it unlikely that 2,4-D can cause cancer in humans.
> Publication Types: * Review* Review, Tutorial PMID: 1820267 [PubMed - indexed for MEDLINE]

We note than an old, but still large review showed that 2,4-D in particular (among chlorophenoxy herbicides and other pesticides) consistently showed a dose/response relation to non-Hodgkin's Lymphoma--i.e. the greater or longer the measured or estimated exposure, the greater the likelihood of acquiring NHL (Sheila Zahm & A. Blair 1992 'Pesticides and non-Hodgkin's Lymphoma' Cancer Research:52:19:5485a-5488a). The following abstract, presumably to a review, seems to us a fair summary of our findings in reviewing this published literature:

> Reuber MD. 1983 Dec 1. Carcinogenicity and toxicity of 2,4-dichlorophenoxy-acetic acid. Sci Total Environ 31:203-18.
> 2,4-Dichlorophenoxyacetic acid (2,4-D) is carcinogenic in male and female rats and probably also in mice. Male and female rats ingesting 2,4-D developed increased incidences of malignant neoplasms. Lymphosarcomas were increased in rats of both sexes, and neoplasms of the mammary gland in female rats. Male rats also had carcinomas of the endocrine organs. 2,4-D isooctyl ester was carcinogenic for the lymphoreticular system in female mice. 2,4-D and 2,4-dichlorophenol also were promoters of neoplasms of the skin in mice. Male mice given 2,4-D isopropyl ester developed an increased incidence of neoplasms of the lung. 2,4-D also is mutagenic and teratogenic in animals and causes poisoning in animals and human beings.
> Publication Types: * Review PMID: 6362003 [PubMed - indexed for MEDLINE]

NEGATIVE CANCER RESULTS/REVIEWS

Crit Rev Toxicol. 2002 Jul;32(4):233-57. Review of 2,4-dichlorophenoxyacetic acid (2,4-D) epidemiology and toxicology. Garabrant DH, Philbert MA. Department of Environmental Health Sciences, University of Michigan School of Public Health, Ann Arbor 48109-2029, USA. dhg@umich.edu
The scientific evidence in humans and animals relevant to cancer risks, neurologic disease, reproductive risks, and immunotoxicity of 2,4-D was reviewed. Despite several thorough in vitro and in vivo animal studies, no experimental evidence exists supporting the theory that 2,4-D or any of its salts and esters damages DNA under physiologic conditions. Studies in rodents demonstrate a lack of oncogenic or carcinogenic effects following a lifetime dietary administration of 2,4-D. Epidemiologic studies provide scant evidence that exposure to 2,4-D is associated with soft tissue sarcoma, non-Hodgkin's lymphoma, Hodgkin's disease, or any other cancer. Overall, the available evidence from epidemiologic studies is not adequate to conclude that any form of cancer is causally associated with 2,4-D exposure. There is no human evidence of adverse reproductive outcomes related to 2,4-D. The available data from animal studies of acute, subchronic, and chronic exposure to 2,4-D, its salts, and esters show an unequivocal lack of systemic toxicity at doses that do not exceed renal clearance mechanisms. There is no evidence that 2,4-D in any of its forms activates or transforms the immune system in animals at any dose. At high doses, 2,4-D damages the liver and kidney and irritates mucous membranes. Although myotonia and alterations in gait and behavioral indices are observed after overwhelming doses of 2,4-D, alterations in the neurologic system of experimental animals are not observed with the administration of doses in the microgram/kg/day range. It is unlikely that 2,4-D has any neurotoxic potential at doses below those required to induce systemic toxicity. Publication Types: * Review PMID: 12184504 [PubMed - indexed for MEDLINE]

Even if your experimental studies had been of the highest quality, we may have identified a critical factor that they ignored (as do all typical chronic toxicology studies, which are designed not to search for almost any toxicity): ***the timing of the dose***. The two studies below find that 2,4-D is <u>not</u> carcinogenic when 2,4-D exposure occurs after weaning, <u>but that it is</u> when the exposure occurs earlier:

> Parfieniuk A, Musiatowicz B, Sulik M. 1993 May 3-10. [Some parameters of Guerin cancer growth after exposure to Pielik (sodium salt of 2,4-dichlorophenoxyacetate)]. Pol Tyg Lek 48:414-6.
> Abstract: Herbicide Pielik (sodium 2,4-dichlorophenoxyacetate) was tested with the aid of Guerin cancer animal model in 129 Wistar rats. An effect of this herbicide on the cancer growth dynamic (size and weight of the tumor), its malignancy (lymphatic nodes involvement), tumor-dependent animal cachexia (real body weight), and survival of rats depending on exposure period have been analysed. Aqueous solution of the herbicide was administered to animals of groups II, IV, V, and VI in the dose of 200 mg/kg body weight daily (1/3 LD50). Young rats were exposed to the herbicide during pre- and postnatal period till the death (groups III, IV and VI in the 80th day of life. Exposure to the herbicide was continued. Rats of all groups were sacrificed in the 16th, 20th, and 42nd day after implantation of Guerin cancer. Eight animals of each group were kept alive to assess survival. **Accelerated growth of the tumor was noted in the animals exposed to the herbicide for the prolonged period of time (before and after birth). The same daily dose administered to the animals after weaning and continued** to the 16th, 20th, and 42nd day of tumor development (group IV) **has not significant effect on tumor growth rate**. An increase in the incidence as well as earlier onset of metastases to auxillary and groin lymphatic nodes were seen in group VI in comparison with the control animals (group III).
> PMID: 8309821 [PubMed - indexed for MEDLINE]

> Sulik M, Matus A, Musiatowicz B, Sulkowska M, Kemona A, Kisielewski W, Sobaniec-Lotowska M, Barwijuk-Machala M. 1996. The effect of a herbicide--sodium salt of 2,4-dichlorophenoxyacetic acid on guerin carcinoma. Rocz Akad Med Bialymst 41:347-62.
> Abstract: The effect of sodium salt of 2,4-dichlorophenoxyacetic acid, being an active component of herbicide "PIELIK", upon the development of Guerin carcinoma implanted in male Wistar rats, was studied. 192 animals were divided in to 6 equal groups: I-animals which obtained physiological salt solution; II-rats exposed to the herbicide in postlactational period; III-animals with Guerin carcinoma, non exposed to the herbicide; IV- rats exposed to the herbicide in postlactational period+Guerin carcinoma; V-animals exposed to the herbicide from prenatal period to the end of an experiment, without Guerin carcinoma; VI-the same as in V group, but with Guerin carcinoma. The effect of the herbicide on tumor growth dynamism (diameters and mass), degree of tumour malignancy (metastases to lymph nodes), animals survival time and morfological changes in the primary tumour and in metastases was evaluated. Basing of the results obtained, it was stated that this herbicide accelerates the development of Guerin carcinoma and reduces the survival time in the rats exposed to it in the prenatal and postnatal period. **<u>However, it does not significantly influence the growth of the carcinoma in the rats exposed only in the postlactational period</u>**.
> PMID: 9020547 [PubMed - indexed for MEDLINE]

POSITIVE RESULTS/IMMUNE CANCERS

Although all cancers involve a failure of the immune system to detect and destroy cancerous cells (proliferating--i.e. uncontrolled replication); the cells of the immune system itself may begin to proliferate and turn cancerous. Over 100 papers in the published literature show that 2,4-D alters the immune system. Thus it is no surprise that so much evidence showing 2,4-D to be carcinogenic to the immune system. So the EPA must weigh especially heavy the extensive published literature on cancer that we summarize below.

It indicates quite overwhelmingly that 2,4-D causes cancer in people and animals and that it is mutagenic and cytogenic (two mechanisms of cancer). The epidemiologic subset associating 2,4-D with cancers of human blood and immune systems is large--and strongly positive according to one recent review (Susan Osburn (ed.) 2001 'Do Pesticides Cause Lymphoma?' Lymphoma Association of America, Chevy Chase MD; 51 pg.).

POSITIVE RESULTS/NON-HODGKIN'S LYMPHOMA (NHL)

Environ Health Perspect. 2003 Nov;111(14):1704-6. Is the decline of the increasing incidence of non-Hodgkin lymphoma in Sweden and other countries a result of cancer preventive measures? Hardell L, Eriksson M. Department of Oncology, University Hospital, Orebro, Sweden. lennart.hardell@orebroll.se
Is the decline of the increasing incidence of non-Hodgkin lymphoma (NHL) in Sweden and other countries a result of cancer preventive measures? The yearly age-standardized incidence of NHL increased significantly in Sweden during 1971-1990, for men an average of 3.2% and for women 3.1%. The corresponding figures for 1991-2000 were -0.8% and -0.2%, respectively. A decline of the increasing incidence has also been seen in other countries, such as the United States, Finland, and Denmark. Immunosuppression is one established risk factor for NHL, possibly with interaction with Epstein-Barr virus. Phenoxyacetic acids and chlorophenols, both pesticides, have been associated with NHL. Use of these chemicals was banned in Sweden in 1977 and 1978, respectively. Also, persistent organic pollutants such as polychlorinated biphenyls, hexachlorobenzene, chlordanes, and dioxins have been shown to increase the risk. Exposure of the whole population occurs predominantly through the food chain. Exposure to such chemicals was highest in the 1960s and 1970s. Because of regulation in the 1970s, exposure has declined substantially in the population. The change in incidence of NHL in Sweden and other countries may serve as a good example of how prohibition and limitation of exposure may be reflected in cancer statistics some decades later. PMID: 14594618 [PubMed - indexed for MEDLINE]

Zahm SH, Weisenburger DD, Babbitt PA, Saal RC, Vaught JB, Cantor KP, Blair A. 1990 Sep . A case-control study of non-Hodgkin's lymphoma and the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) in eastern Nebraska. Epidemiology 1:349-56.
Abstract: To evaluate the role of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) in the development of non-Hodgkin's lymphoma (NHL), we conducted a population-based, case-control study in 66 counties in eastern Nebraska. Telephone interviews were conducted with 201 white men diagnosed with NHL between July 1, 1983, and June 30, 1986, and with 725 controls. There was a 50% excess of NHL among men who mixed or applied 2,4-D (odds ratio [OR] = 1.5; 95% confidence interval = 0.9, 2.5). The risk of NHL increased with the average frequency of use to over threefold for those exposed 20 or more days per year (p for trend = 0.051). Adjusting for use of organophosphate insecticides lowered the risk estimate for frequent users (OR = 1.8), but adjustment for fungicide use increased the risk estimate (OR = 4.5). Simultaneous adjustment for organophosphates and fungicides yielded an OR of 3.1 for farmers who mixed or applied 2,4-D more than 20 days per year. Risk also increased with degree of exposure, as indicated by application method and time spent in contaminated clothing, but not with the number of years of 2,4-D use or failure to use protective equipment. Although other pesticides, especially organophosphate insecticides, may be related to NHL, the risk associated with 2,4-D does not appear to be explained completely by these other exposures.

Cantor KP, Blair A, Everett G, Gibson R, Burmeister LF, Brown LM, Schuman L, Dick FR. 1992. Pesticides and other agicultural risk factors for Non-Hodgkin's lymphoma among men in Iowa and Minnesota. Cancer Res 52:2447-2455.
Abstract: Data from an in-person interview study of 622 white men with newly diagnosed non-Hodgkin's lymphoma and 1245 population-based controls in Iowa and Minnesota were used to measure the risk associated with farming occupation and specific agricultural exposures. Men who ever farmed were at slightly elevated risk of non-Hodgkin's lymphoma (odds ratio = 1.2, 95% confidence interval = 1.0-1.5) that was not linked to specific crops or particular animals. Elevated risks were found, with odds ratio generally 1.5-fold or greater, for personal handling, mixing, or application of several pesticide groups and for individual insecticides, including carbaryl, chlordane, dichlorodiphenyltrichloroethane, diazinon, dichlorvos, lindane, malathion, nicotine and toxaphene. Associations were generally stronger for first use prior to 1965 than more recently, and when protective clothing or equipment was not used. Small risks were associated with the use of the phenoxyacetic acid herbicide 2,4-dichlorophenoxyacetic acid, but the risks did not increase with latency of failure to use protective equipment. Exposure to numerous pesticides poses problems of interpreting risk associated with a particular chemical, and multiple comparisons increase the chances of false-positive findings. In contrast nondifferential exposure misclassification due to inaccurate recall can bias risk estimates toward the null and mask positive associations. In the face of these methodological and statistical issues, the consistency of several finding, both within this study and

with observations of others, suggests an important role for several insecticides in the etiology on non-Hodgkin's lymphoma among farmers.
Keywords:

Fontana A, Picoco C, Masala G, Prastaro C, Vineis P. 1998. Incidence rates of lymphomas and environmental measurements of phenoxy herbicides: ecological analysis and case-control study. Arch Environ Health 53:384-387.
Abstract: The authors conducted an ecological study of the distribution of malignant lymphomas in a rice-growing area in northern Italy. They considered data on concentrations of phenoxy herbicides in soil and water and found the highest incidence of non-Hodgkin's lymphoma in subjects who lived in an area where 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid existed in very high concentrations. During 1985-1988, the incidence of non-Hodgkin's lymphoma in males in the most-polluted municipalities was twice as high as was noted for the remaining less-polluted territories. During 1991-1993, non-Hodgkin's lymphoma was higher by 60%. The authors also conducted a population-based case-control study. They found an association between employment of women in rice-growing jobs (particularly as rice weeders) and risk of non-Hodgkin's lymphoma (odds ratio = 1.9; 95% confidence interval = 0.6, 6.0). Work in rice fields was correlated strongly with residence in polluted areas. The authors did not detect an association between area of residence or occupation and incidence of Hodgkin's disease.
Keywords:

Cancer. 1999 Mar 15;85(6):1353-60. Comment in: * Cancer. 1999 Aug 15;86(4):729-31.
A case-control study of non-Hodgkin lymphoma and exposure to pesticides. Hardell L, Eriksson M. Department of Oncology, Orebro Medical Center, Sweden.
BACKGROUND: The incidence of non-Hodgkin lymphoma (NHL) has increased in most Western countries during the last few decades. Immunodefective conditions are established risk factors. In 1981, the authors reported an increased risk for NHL following exposure to certain pesticides. The current study was designed to further elucidate the importance of phenoxyacetic acids and other pesticides in the etiology of NHL. METHODS: A population-based case-control study in northern and middle Sweden encompassing 442 cases and twice as many controls was performed. Exposure data were ascertained by comprehensive questionnaires, and the questionnaires were supplemented by telephone interviews. In total, 404 cases and 741 controls answered the questionnaire. Univariate and multivariate analyses were performed with the SAS statistical data program. RESULTS: Increased risk for NHL was found for subjects exposed to herbicides (odds ratio [OR], 1.6; 95% confidence interval [CI], 1.0-2.5) and fungicides (OR, 3.7; 95% CI, 1.1-13.0). Among herbicides, the phenoxyacetic acids dominated (OR, 1.5; 95% CI, 0.9-2.4); and, when subclassified, one of these, 4-chloro-2-methyl phenoxyacetic acid (MCPA), turned out to be significantly associated with NHL (OR, 2.7; 95% CI, 1.0-6.9). For several categories of herbicides, it was noted that only exposure during the most recent decades before diagnosis of NHL was associated with an increased risk of NHL. Exposure to impregnating agents and insecticides was, at most, only weakly related to NHL. CONCLUSIONS: Exposure to herbicides in total, including phenoxyacetic acids, during the decades before NHL diagnosis resulted in increased risk for NHL. Thus, the risk following exposure was related to the latency period. Fungicides also increased the risk for NHL when combined, but this group consisted of several different agents, and few subjects were exposed to each type of fungicide. PMID: 10189142 [PubMed - indexed for MEDLINE]

Hardell L, Eriksson M, Degerman A. 1994 May 1. Exposure to phenoxyacetic acids, chlorophenols, or organic solvents in relation to histopathology, stage, and anatomical localization of non-hodgkins lymphoma. Cancer Res 54:2386-2389.
Abstract: Results on 105 cases with histopathologically confirmed non-Hodgkin's lymphoma (NHL) and 335 controls from a previously published case-control study on malignant lymphoma are presented together with some extended analyses. No occupation was a risk factor for NKL. Exposure to phenoxyacetic acids yielded, in the univariate analysis, an odds ratio of 5.5 with a 95% confidence interval of 2.7-11. Most cases and controls were exposed to a commercial mixture of 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid. Exposure to chlorophenols gave an odds ratio of 4.8 (2.7-8.8) with pentachlorophenol being the most common type. Exposure to organic solvents yielded an odds ratio of 2.4 (1.4-3.9). These results were not significantly changed in the multivariate analysis. Dichlorodiphenyltrichloroethane, asbestos, smoking, and oral snuff were not associated with an increased risk for NHL. The results regarding increased risk for NHL following exposure to phenoxyacetic acids, chlorophenols, or organic solvents were not affected by histopathological type, disease stage, or anatomical site of disease presentation. Median survival was somewhat longer in cases exposed to organic solvents than the rest. This was explained by more prevalent exposure to organic solvents in the group of cases with good prognosis NHL histopathology. [References: 29] Number of References 29
Keywords:

Br J Ind Med. 1981 Feb;38(1):27-33. Soft-tissue sarcomas and exposure to chemical substances: a case-referent study. Eriksson M, Hardell L, Berg NO, Moller T, Axelson O. In 1977 several patients were seen with soft-tissue sarcomas and previous exposure to phenoxy acids. This clinical observation resulted in a cases-referent (case-control) study being undertaken which showed that exposure to phenoxy acids or chlorophenols, which are chemically related, gave a roughly six-fold increase in the risk for this type of tumour. A further case-referent study of soft-tissue sarcomas has now been performed to confirm these earlier findings and also to obtain further information on the effects of different phenoxy acids. This new investigation gave an increase of the same magnitude in the risk for soft-tissue sarcomas after exposure to

phenoxy acids or chlorophenols, but this risk related also to exposure to phenoxy acids free from impurities, such as polychlorinated dibenzodioxins and dibenzofurans. PMID: 7470401 [PubMed - indexed for MEDLINE]

McDuffie HH, Pahwa P, McLaughlin JR, Spinelli JJ, Fincham S, Dosman JA, Robson D, Skinnider LF, Choi NW. 2001 Nov. Non-hodgkin's lymphoma and specific pesticide exposures in men: cross-canada study of pesticides and health. Cancer Epidemiology, Biomarkers & Prevention 10:1155-1163.
Abstract: Our objective in the study was to investigate the putative associations of specific pesticides with non-Hodgkin's Lymphoma [NHL; International Classification of Diseases, version 9 (ICD-9) 200, 202]. We conducted a Canadian multicenter population-based incident, case (n = 517)-control (n = 1506) study among men in a diversity of occupations using an initial postal questionnaire followed by a telephone interview for those reporting pesticide exposure of 10 h/year or more, and a 15% random sample of the remainder. Adjusted odds ratios (ORs) were computed using conditional logistic regression stratified by the matching variables of age and province of residence, and subsequently adjusted for statistically significant medical variables (history of measles, mumps, cancer, allergy desensitization treatment, and a positive history of cancer in first-degree relatives). We found that among major chemical classes of herbicides, the risk of NHL was statistically significantly increased by exposure to phenoxyherbicides [OR, 1.38; 95% confidence interval (CI), 1.06-1.81] and to dicamba (OR, 1.88; 95% CI, 1.32-2.68). Exposure to carbamate (OR, 1.92; 95% CI, 1.22-3.04) and to organophosphorus insecticides (OR, 1.73; 95% CI, 1.27-2.36), amide fungicides, and the fumigant carbon tetrachloride (OR, 2.42; 95% CI, 1.19-5.14) statistically significantly increased risk. Among individual compounds, in multivariate analyses, the risk of NHL was statistically significantly increased by exposure to the herbicides 2,4-dichlorophenoxyacetic acid (2,4-D; OR, 1.32; 95% CL 1.01-1.73), mecoprop (OR, 2.33; 95% CI, 1.58-3.44), and dicamba (OR, 1.68; 95% CI, 1.00-2.81); to the insecticides malathion (OR, 1.83; 95% CI, 1.31-2.55), 1,1,1-trichloro-2,2-bis (4-chlorophenyl) ethane (DDT), carbaryl (OR, 2.11; 95% CI, 1.21-3.69), aldrin, and lindane; and to the fungicides captan and sulfur compounds. In additional multivariate models, which included exposure to other major chemical classes or individual pesticides, personal antecedent cancer, a history of cancer among first-degree relatives, and exposure to mixtures containing dicamba (OR, 1.96; 95% CL 1.40-2.75) or to mecoprop (OR, 2.22; 95% CL 1.49-3.29) and to aldrin (OR, 3.42; 95% CI, 1.18-9.95) were significant independent predictors of an increased risk for NHL, whereas a personal history of measles and of allergy desensitization treatments lowered the risk. We concluded that NHL was associated with specific pesticides after adjustment for other independent predictors. [References: 47] Number of References 47
Keywords:

Vineis P, Faggiano F, Tedeschi M, Ciccone G. 1991 Mar 6. Incidence rates of lymphomas and soft-tissue sarcomas and environmental measurements of phenoxy herbicides. J Natl Cancer Inst 83:362-3. [ABSTRACT: FOUND SIGNIFICANT ASSOCTN. NHL W/ AREAS OF HIGH SOIL & WATER 2,4-D LEVELS VS. LOW LEVELS:
Weisenburger DD. 1990. Environmental epidemiology of non-Hodgkin's lymphoma in eastern Nebraska. Am J Ind Med 18:303-5.
Abstract: The incidence of non-Hodgkin's lymphoma (NHL) is increased in many counties in eastern Nebraska. Histologic analysis has revealed a twofold increase in the clinically aggressive, diffuse large cell subtype of NHL. To investigate the possible association between NHL and agricultural exposures, a population-based case-control study was conducted in eastern Nebraska in 1985. Telephone interviews were conducted with 201 men having histologically confirmed NHL and 725 controls. Among men, the use of the herbicide 2,4-D was associated with a 50% increased risk of NHL (OR 1.5, 95% CI 0.9, 2.4). Personal exposure to 2,4-D more than 20 days per year increased the risk threefold (OR 3.3, 95% CI 0.5, 22.1). Several classes of insecticides were also associated with increased risk: organophosphates (OR 1.9, 95% CI 1.1, 3.1), carbamates (OR 1.8, 95% CI 1.0, 3.2), and chlorinated hydrocarbons (OR 1.4, 95% CI 0.8, 2.3). As a result of intense agrichemical use, extensive contamination of shallow groundwater by nitrate and atrazine has also occurred in eastern Nebraska. A twofold increased incidence of NHL is present in counties with greater than 20% of the wells contaminated by nitrate (greater than 10 ppm) and in counties with intense fertilizer use. These findings suggest that NHL in eastern Nebraska may be related to the use of pesticides and nitrogen fertilizers.

Wiklund K, Lindefors BM, Holm LE. 1988 Jan. Risk of malignant lymphoma in Swedish agricultural and forestry workers. Br J Ind Med 45:19-24.
Abstract: The risk of malignant lymphoma after possible exposure to phenoxy acid herbicides was studied in 354,620 Swedish men who, according to a national census in 1960, were employed in agriculture or forestry. The cohort was divided into subcohorts according to assumed exposure and compared with 1,725,645 Swedish men having other economic activities. All were followed up in the Cancer-Environment Register between 1961 and 1979. Non-Hodgkin lymphoma was found in 861 men in the study cohort. The relative risk was not significantly increased in any subcohort, did not differ significantly between the subcohorts, and showed no time related increase in the total cohort or any subcohort. Hodgkin's disease was found in 355 men in the study cohort. Relative risks significantly higher than unity were found among fur farming and silviculture workers where the relative risks were 4.45 and 2.26, respectively. All five cases in the former group were engaged in mink farming. A time related rising trend in relative risk was found in the silviculture subcohort. Elsewhere the relative risk did not diverge from unity and no time related trend was discernible.

Zahm SH, Weisenburger DD, Babbitt PA, Saal RC, Vaught JB, Cantor KP, Blair A. 1990. A Case-Control Study of Non-

Hodgkin's Lymphoma and the Herbicide 2,4-Dichlorophenoxyacetic Acid (2,4-D) in Eastern Nebraska. Epidemiology 1:349-356.

Abstract: To evaluate the role of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) in the development of non-Hodgkin's lymphoma (NHL), we conducted a population-based, case-control study in 66 counties in eastern Nebraska. Telephone interviews were conducted with 201 white men diagnosed with NHL between July 1, 1983, and June 30, 1986, and with 725 controls. There was a 50% excess of NHL among men who mixed or applied 2,4-D (odds ratio [OR] = 1.5; 95% confidence interval = 0.9, 2.5). The risk of NHL increased with the average frequency of use to over threefold for those exposed 20 or more days per year (p for trend = 0.051). Adjusting for use of organophoshate insecticides lowered the risk estimate (OR = 4.5). simultaneous adjustment for organophosphates and fungicides yielded an OR of 3.1 for farmers who mixed or applied 2,4-D more that 20 days per year. Risk also increased with degree of exposure, as indicated by application method and time spent in contaminated clothing, but not with the number of years of 2,4-D use or failure to use protective equipment. Although other pesticides, especially organophosphate insecticides, may be related to NHL, the risk associated with 2,4-D does not appear to be explained completely by these other exposures.Keywords:

Scand J Work Environ Health. 1994 Feb;20(1):42-7.

Non-Hodgkin's lymphoma and agricultural practices in the prairie provinces of Canada.

Morrison HI, Semenciw RM, Wilkins K, Mao Y, Wigle DT.
Bureau of Chronic Disease Epidemiology, Laboratory Centre for Disease Control, Health Canada, Ottawa.

OBJECTIVES--The aim of this study was to provide an update of a cohort study (1971-1985) that previously reported a significant trend in the risk of non-Hodgkin's lymphoma among male Saskatchewan farm operators according to fuel-oil expenditures and herbicide spraying for farms less than 1000 acres (2570 hectares) by including two additional Canadian prairie provinces, two additional years of follow-up, and data from the 1981 Census of Agriculture. METHODS--Information on farmers from 1971 records of the Census of Agriculture was linked to 1971 records of the Census of Population, to 1981 records of the Census of Agriculture, and to death records. Poisson regression was used to estimate risks according to herbicide spraying and fuel and oil expenditures. RESULTS--The addition of a further two years of follow-up resulted in lower risk estimates associated with herbicide spraying for Saskatchewan. No excess risk was observed between herbicide spraying and non-Hodgkin's lymphoma for Alberta or Manitoba in the 1971 data. However, a significantly increased risk of non-Hodgkin's lymphoma according to acres sprayed with herbicides was observed for the three provinces combined when the herbicide spraying data from the 1981 Census of Agriculture was used [> or = 380 acres (> or = 939 hectares) sprayed, rate ratio 2.11, 95% confidence interval 1.1-3.9]. CONCLUSIONS--Although the current results are not entirely consistent with the original Saskatchewan analysis, they support the overall finding of an association between herbicides and risk of fatal non-Hodgkins lymphoma. Prospective cohort studies are needed to overcome the limitations of existing epidemiologic studies.
PMID: 8016598 [PubMed - indexed for MEDLINE]

Med Lav. 1990 Nov-Dec;81(6):499-505.

Mortality study of Canadian male farm operators: cancer mortality and agricultural practices in Saskatchewan.

Ritter L, Wigle DT, Semenciw RM, Wilkins K, Riedel D, Mao Y.
Health Protection Branch, Health and Welfare Canada, Ottawa, Ontario.

The present investigation involved an analysis of approximately 70,000 male Saskatchewan farm operators, a subset of the 365,000 Canadian farm operators to be investigated in the Canadian Farm Operator Mortality Study. The results of the Saskatchewan analysis indicate that during the interval studied, overall mortality among Saskatchewan farmers was 25% lower than that for all Saskatchewan men, and that, during the same time interval, the risk of death from all types of cancer was also about 25% lower among Saskatchewan farmers than to all Saskatchewan men. Although the present study indicates that overall mortality of death from cancer was 25% lower among Saskatchewan male farmers, there was a relationship between non-Hodgkin's lymphoma mortality and acres sprayed for weeds; a similar risk relationship between expenditures on fuel oil and risk of death from non-Hodgkin's lymphoma was also evident. The magnitude of risk for Saskatchewan farmers is probably greater than that reflected in the estimates in this study, due to the likelihood of misclassification of exposure. There is a particular need for further studies in this area to improve the quantification of farming-related exposures, and to study the exposure history of individuals who develop non-Hodgkin's lymphoma. PMID: 2100765 [PubMed - indexed for MEDLINE]

POSITIVE RESULTS/NHL ANALOGUE: CANINE MALIGNANT LYMPHOMA (CML)

In addition to falsely denigrating and ignoring the quality of peer review (including post-publication) in independent journals, The Industry Task Force is strangely silent about the this 1995 follow-up to the 1991 Hayes et al. CML study.

Hayes HM, Tarone RE, Cantor KP. 1995. On the association between canine malignant lymphoma and opportunity for exposure to 2,4-dichlorophenoxyacetic acid. Environ Res 70:119-125.

Abstract: In response to criticisms raised regarding a case-control study of canine malignant lymphoma, the results of several ancillary analyses are reported. The case-control study demonstrated a significant association between risk for canine malignant lymphoma and the opportunity for exposure to 2,4-dichlorophenoxyacetic acid herbicides. It is demonstrated that risk estimates do not vary by type of control group (i.e., tumor control or nontumor control group), by method of response (i.e., self-administered or telephone interview), or by geographic area. Questions related to the potential for referral bias, supposed inconsistencies in subject responses regarding frequency of herbicide use, and ambiguities regarding exposure classification are also examined.
Keywords:

Hayes HM, Tarone RE, Cantor KP, Jessen CR, McCurnin DM, Richardson RC. 1991. Case-control study of canine malignant lymphoma: Positive association with dog owner's use of 2,4-dichlorophenoxyacetic acid herbicides. J Natl Cancer Inst 83:1226-1231.
Abstract: A hospital-based case-control study of companion dogs examined the risk of developing canine malignant lymphoma associated with the use of chemicals in the home. The present study suggests that human health implications of 2,4-D exposure in the home environment should receive further investigation.Keywords:

Sternberg SS. 1992 Feb 19. Canine malignant lymphoma and 2,4-dichlorophenoxyacetic acid herbicides. J Natl Cancer Inst 84:271. [letter? get it..]

In addition, the following study strongly supports 2,4-D carcinogenicty to pets in close contact with it; such exposure is proven by the study after it:

J Am Vet Med Assoc. 2004 Apr 15;224(8):1290-7. Herbicide exposure and the risk of transitional cell carcinoma of the urinary bladder in Scottish Terriers. Glickman LT, Raghavan M, Knapp DW, Bonney PL, Dawson MH.
Department of Veterinary Pathobiology, School of Veterinary Medicine, Purdue University, West Lafayette, IN 47907-2027, USA.
OBJECTIVE: To determine whether exposure to lawn or garden chemicals was associated with an increased risk of transitional cell carcinoma (TCC) of the urinary bladder in Scottish Terriers. DESIGN: Case-control study. ANIMALS: 83 Scottish Terriers with TCC (cases) and 83 Scottish Terriers with other health-related conditions (controls). PROCEDURE: Owners of study dogs completed a written questionnaire pertaining to exposure to lawn or garden chemicals during the year prior to diagnosis of TCC for case dogs and during a comparable period for control dogs. RESULTS: The risk of TCC was significantly increased among dogs exposed to lawns or gardens treated with both herbicides and insecticides (odds ratio [OR], 7.19) or with herbicides alone (OR, 3.62), but not among dogs exposed to lawns or gardens treated with insecticides alone (OR, 1.62), compared with dogs exposed to untreated lawns. Exposure to lawns or gardens treated with phenoxy herbicides (OR, 4.42) was associated with an increased risk of TCC, compared with exposure to untreated lawns or gardens, but exposure to lawns or gardens treated with nonphenoxy herbicides (OR, 3.49) was not significantly associated with risk of TCC. CONCLUSIONS AND CLINICAL RELEVANCE: Results suggest that exposure to lawns or gardens treated with herbicides was associated with an increased risk of TCC in Scottish Terriers. Until additional studies are performed to prove or disprove a cause-and-effect relationship, owners of Scottish Terriers should minimize their dogs' access to lawns or gardens treated with phenoxy herbicides. PMID: 15112777 [PubMed - indexed for MEDLINE]

Reynolds PM, Reif JS, Ramsdell HS, Tessari JD. 1994 Apr-May. Canine exposure to herbicide-treated lawns and urinary excretion of 2,4-dichlorophenoxyacetic acid. Cancer Epidemiol Biomarkers Prev 3:233-7.
Abstract: A recent study by Hayes et al. (J. Natl. Cancer. Inst., 83: 1226-1231, 1991) found an increased risk of malignant lymphoma associated with exposure to 2,4-dichlorophenoxyacetic acid (2,4-D) in pet dogs. We conducted a study to determine the extent to which dogs absorb and excrete 2,4-D in urine after contact with treated lawns under natural conditions. Among 44 dogs potentially exposed to 2,4-D-treated lawns an average of 10.9 days after application, 2,4-D concentrations greater than or equal to 10.0 micrograms/l were found in 33 dogs (75%) and concentrations of > or = 50 micrograms/l were found in 17 (39%). Among 15 dogs with no known exposure to a 2,4-D-treated lawn in the previous 42 days, 4 (27%) had evidence of 2,4-D in urine, 1 at a concentration of > or = 50 micrograms/l. The odds ratio for the association between exposure to a 2,4-D-treated lawn and the detection of > or = 50 micrograms/l 2,4-D in urine was 8.8 (95% confidence interval, 1.4-56.2). Dogs exposed to lawns treated within 7 days before urine collection were more than 50 times as likely to have 2,4-D at concentrations > or = 50 micrograms/l than dogs with exposure to a lawn treated more than 1 week previously (odds ratio = 56.0; 95% confidence interval, 10.0-312.2). The highest mean concentration of 2,4-D in urine (21.3 mg/l) was found in dogs sampled within 2 days after application of the herbicide.(ABSTRACT TRUNCATED AT 250 WORDS)

Study finds higher rates of bladder cancer among dogs exposed to herbicides
Thursday, March 03, 2005 Suzanne Hively Plain Dealer Columnist shively@plaind.com, 216-999-4554
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"Eat your veggies!" applies to dogs too. A study at Purdue University in West Lafayette, Ind., revealed Scottish terriers that ate vegetables three times a week reduced their risk of cancer of the urinary bladder by 50 percent, or about the same as humans who ate vegetables.

Green leafy vegetables provided about a 90 percent reduction in risk of cancer; yellow vegetables, 70 percent; and cruciferous vegetables, about 80 percent, said Lawrence Glickman, veterinarian and researcher at Purdue.

The findings are part of a study that examined Scottish terriers and incidences of cancer of the urinary bladder (transitional cell carcinoma) caused by exposure to chemically treated lawns.

Scottish terriers were used for the test because they have an incidence of bladder cancer 20 times greater than any other breed of dogs, said Glickman.

Owners of 83 Scottish terriers with bladder cancer and 83 with other health-related conditions completed a written questionnaire pertaining to exposure to lawn or garden chemicals during the year before diagnosis of the cancer for case dogs and a comparable period for control dogs.

The risk of bladder cancer was significantly increased among Scotties exposed to lawns or gardens treated with both herbicides and insecticides or with herbicides only.

Scotties exposed to lawns or gardens treated with insecticides alone had a small but not significantly increased risk of bladder cancer compared with dogs exposed to untreated lawns.

The prevalence of bladder cancer in dogs examined at veterinary teaching hospitals in North America increased by more than 600 percent between 1975 and 1995, according to the Journal of the American Veterinary Medical Association.

Scottish terriers, Shetland sheepdogs, wirehaired fox terriers and West Highland white terriers had a significantly increased risk of bladder cancer, compared with mixed-breed dogs. This suggests a genetic predisposition to bladder cancers in terriers and primarily in Scottish terriers.

Glickman and colleagues found the risk of bladder cancer was higher among dogs exposed to phenoxy acid herbicides, the most commonly used chemical in agriculture, or nonphenoxy acid herbicides, compared with dogs exposed to lawns or gardens that did not receive an herbicide application.

Phenoxy acid is an active ingredient in 2,4-D, a commonly used herbicide. The Environmental Protection Agency says 2,4-D is safe.

Glickman said what goes on in the laboratory and the lawn are two different things. The EPA doesn't require inert ingredients in lawn products to be listed on the label, he said.

"Those inert ingredients are cancer-causing chemicals," Glickman said. "We know they contain heavy metals, petroleum-based solvents and cadmium.

"We can't do a study because the labels don't tell us what is in the product."

A follow-up study at Purdue will examine exposure to lawn chemicals in dogs and children.

When chemicals are applied to a lawn, it is generally recommended that people and pets stay off the lawn for 24 hours, Glickman said.

"We are studying 25 homes with lawns treated with herbicides and collecting grass clippings before application, 24 hours after and three

days after, he said.

"We are looking at the chemicals found, if they change over a period of time and if they are still present beyond 24 hours."

Urine samples are being collected from children and dogs in these households. All breeds of dogs are included in the study.

The study will determine if children and dogs exposed to the lawn get these chemicals in their systems, how high the concentrations are and the nature of exposure beyond three days.

A control group in which lawns have not been chemically treated is included in the study.

"We are going to look at chemicals to see if there are known cancer-causing agents," Glickman said. "If we find chemicals for which there is little information, we will conduct tests to see if they are possibly cancer-causing agents."

POSITIVE RESULTS/MULTIPLE MYELOMA

Am J Ind Med. 1992;22(3):305-12. Malignant lymphoproliferative diseases in occupations with potential exposure to phenoxyacetic acids or dioxins: a register-based study. Eriksson M, Hardell L, Malker H, Weiner J. Department of Oncology, University Hospital, Umea, Sweden. The Swedish Cancer Environment Register (CER) is a linkage of census data (e.g., on occupations) with the Swedish Cancer Register. It has been used in different studies to generate hypotheses on occupational risk factors for malignant tumors. In this study the risk for malignant lymphoma and multiple myeloma in occupations with potential exposure to phenoxyacetic acids or other related substances were investigated. An increased standardized incidence ratio (SIR) of 1.3 for multiple myeloma was verified in farmers (no. of cases = 335). This finding applied to both sexes, and the SIR increased over successive time periods. Regarding malignant lymphoma an increased SIR of 1.2 was found in farmers (no. = 227) for the latest time period studied (i.e. 1979-1984). When non-Hodgkin's lymphoma was studied separately, an increased risk (SIR = 1.2) was found only in carpenters (no. = 149), whereas for Hodgkin's disease, sawmill workers (no. = 10) had an increased SIR of 2.1. Physicians also had an elevated risk for malignant lymphoma. A major shortcoming in register studies such as CER is that no individual exposure data on different agents are available. Lack of an association between an occupation and a specific malignant disease, therefore, may not be taken as evidence that persons within that occupation are not at increased risk for that disease. PMID: 1519615 [PubMed - indexed for MEDLINE]

POSITIVE RESULTS/LEUKEMIA

Brown LM, Blair A, Gibson R, Everett GD, Cantor KP, Schuman LM, Burmeister LF, Van Lier SF, Dick F. 1990 Oct 15. Pesticide exposures and other agricultural risk factors for leukemia among men in Iowa and Minnesota. Cancer Res 50:6585-91.
Abstract: Mortality surveys and death certificate studies have suggested an association between leukemia and farming. To investigate whether exposure to carcinogens in an agricultural setting is related to risk of leukemia, the authors conducted a population-based case-control interview study of 578 white men with leukemia and 1245 controls living in Iowa and Minnesota. Consistent with recent mortality studies, there were slight, but significant, elevations in risk for all leukemia [odds ratio (OR) 1.2] and chronic lymphocytic leukemia (OR 1.4) for farmers compared to nonfarmers. There were no significant associations with leukemia for exposure to specific fungicides, herbicides (including 2,4-D and 2,4,5-T), or crop insecticides. However, significantly elevated risks for leukemia of greater than or equal to 2.0 were seen for exposure to specific animal insecticides including the organophosphates crotoxyphos (OR 11.1), dichlorvos (OR 2.0), and famphur (OR 2.2) and the natural product pyrethrins (OR 3.7) and the chlorinated hydrocarbon methoxychlor (OR 2.2). There were also smaller, but significant, risks associated with exposure to nicotine (OR 1.6) and DDT (OR 1.3). This finding of elevated risks for insecticides used on animals deserves further evaluation.

Schreinemachers DM. 2000 Sep. Cancer mortality in four northern wheat-producing states. Environ Health Perspect 108:873-881.
Abstract: Chlorophenoxy herbicides are used both in cereal grain agriculture and in nonagricultural settings such as right-of-ways, lawns, and parks. Minnesota, North Dakota, South Dakota, and Montana grow most of the spring and durum

wheat produced in the United States. More than 90% of spring and durum wheat is treated with chlorophenoxy herbicides, in contrast to treatment of approximately 30% of winter wheat. In this ecologic study I used wheat acreage as a surrogate for exposure to chlorophenoxy herbicides. I investigated the association of chlorophenoxy herbicides with cancer mortality during 1980-1989 for selected counties based on level of agriculture (greater than or equal to 20%) and rural population (greater than or equal to 50%). Age-standardized cancer mortality rates were determined for grouped counties based on tertiles of wheat acreage per county or for individual counties for frequently occurring cancers. The cancer sites that showed positive trends of increasing cancer mortality with increasing wheat acreage were esophagus, stomach, rectum, pancreas, larynx, prostate, kidney and meter, brain, thyroid, bone, and all cancers (men) and oral cavity and tongue, esophagus, stomach, liver and gall bladder and bile ducts, pancreas, cervix, ovary, bladder, and other urinary organs, and all cancers (women). Rare cancers in men and women and cancers in boys and girls were studied by comparing counties above and below the median of wheat acreage per county. There was increased mortality for cancer of the nose and eye in both men and women, brain and leukemia in both boys and girls, and all cancers in boys. These results suggest an association between cancer mortality and wheat acreage in counties of these four states. [References: 52] Number of References 52 Keywords:

Swaen GMH, van Amelsvoort LGPM, Slangen JJM, Mohren DCL. 2004 May. Cancer mortality in a cohort of licensed herbicide applicators. International Archives of Occupational & Environmental Health 77:293-295.
Abstract: Objectives. In order to expand our knowledge on the possible long-term health effects of exposure to herbicides, we updated the follow-up of a cohort of 1,341 licensed herbicide applicators in the Netherlands. The earlier report indicated that there might be an increased risk for multiple myeloma in this group. Although that finding was statistically significant, the result was based on a small number of cases. Methods. We expanded the follow-up from 1 January 1988 to 1 January 2001, which added 13 years to the follow-up. We now report on the causes of death of 196 exposed workers. Results. Our findings indicate that licensed herbicide applicators were at an increased risk for skin cancer mortality [standardized mortality ratio (SMR)=357.4, 95% confidence interval (CI) 115.1-827.0]. It is not clear if this excess of skin cancer should be attributed to herbicide exposure or to excess exposure to sunlight. [References: 12] Number of References 12Keywords:

POSITIVE RESULTS/SOFT TISSUE SARCOMAS (STS); BRAIN; AND OTHER **NON**-IMMUNE CANCERS

BRAIN CANCER

The authors of this un-translated paper consistently find correlate phenoxy herbicides with cancers, so perhaps this study does; and being relatively recent it may focus on 2,4-D:

Lakartidningen. 1997 Feb 26;94(9):728-31. [Increased incidence of brain tumors. A study of Swedish children and adolescents aged 0-19] [Article in Swedish] Hardell L, Tondel M, Flodin U, Skoldestig A, Axelson O, Jakobsson S, Eriksson M, Carlsson G. Onkologiska kliniken, Regionsjukhuset, Orebro. PMID: 9091748 [PubMed - indexed for MEDLINE]

...The chronic tox. study done for 2,4-D registration, finding astrocytomas in (male only?) rats.

When administered in rabbits' drinking water, the sodium salt of 2,4-D caused an increase in the number of chromosomes, **brain cells with too many chromosomes** and cells with multiple chromosome sets: K. Atanassov 1992 'Effect of the herbicide Schpritshormit' (salt in 2,4-D) Animal Science 29:54-61.
[ALSO LISTED IN 'POSITIVE RESULTS/MUTAGENICITY']

Brusco A, Saavedra JP, Garcia G, Tagliaferro P, Deduffard AME, Duffard R. 1997 Apr. 2,4-dichlorophenoxyacetic acid through lactation induces astrogliosis in rat brain. Molecular & Chemical Neuropathology 30:175-185.
Abstract: Comparison of astroglial immunoreactivity in mesencephalon, cerebellum, and hippocampus of 25-d-old rat pups exposed to 2,4-dichlorophenoxyacetic acid (2,4-D) through the mother's milk was made using a quantitative immunohistochemical analysis. A glial reaction was detected at the level of serotonergic nuclei and extreme astrogliosis in the hippocampus and cerebellum. A quantitative analysis of reactive astrocytes was performed by using GFAP and S-100 protein as specific markers. The study showed a significant increase in their number, size, number of processes, and density of immunostaining in 2,4-D-exposed animals. Exposure to 2,4-dichlorophenoxyacetic acid on the first days of life modifies the astroglial cytoarchitecture in parallel to previously described neuronal changes. [References: 28] Number of References 28 Keywords:

Garcia G, Tagliaferro P, Bortolozzi A, Madariaga MJ, Brusco A, de Duffard AME, Duffard R, Saavedra JP. 2001 Dec. Morphological study of 5-ht neurons and astroglial cells on brain of adult rats perinatal or chronically exposed to 2,4-

dichlorophenoxyacetic acid. Neurotoxicology 22:733-741.
Abstract: 2,4-D is a chlorophenoxyherbicide used worldwide. We have studied the morphological alterations of 5-HT neurons and glial cells in the mesencephalic nuclei of adult rats exposed to 2,4-D both perinatally (during pregnancy, and lactation) and chronically, (during pregnancy,, lactation and after weaning) with quantitative methods. pregnant rats were daily, exposed to 70 mg/kg of 2,4-D from gestation day, (GD) 16 to post-natal day, (PND) 23 through diet. After weaning, pups were assigned to one of two sub-groups: T1 (fed with untreated diet until PND 90) and T2 (maintained with 2,4-D diet until PND 90). Brain sections were immunocytochemically, stained using poly,clonal anti-5-HT anti-GFAP and anti-S-100 protein antibodies as cells markers. 2,4-D exposure during pregnancy and lactancy, (T1 group) produced an increase in 5-HT neuronal area and immunoreactivity (IR) in the mesencephalic nuclei studied. However, with the chronical 2,4-D exposure (T2 group) only, the 5-HT neuronal area from the dorsal raphe nucleus (DRN) was increased, suggesting an adaptable response of 5-HT neurons in median raphe nucleus (MRN). The presence of reactive astrocytes in mesencephalic nuclei and in hippocampus were also different for the two 2,4-D exposure designs, showing the existence of a correspondence between neuronal changes and astrogliosis. Results support evidences that 2,4-D alters the serotoninergic system and that 5-HT neurons of each mesencephalic nuclei show different responses to the 2,4-D exposure designs which are parallel to astrogliosis. (C) 2001 Elsevier Science Inc. All rights reserved. [References: 55]
Number of References 55 Keywords:

POSITIVE RESULTS/STS AND UNSPECIFIED CANCERS:

JAMA. 1986 Sep 5;256(9):1141-7. Erratum in: * JAMA 1986 Dec 26;256(24):3351.
Agricultural herbicide use and risk of lymphoma and soft-tissue sarcoma. Hoar SK, Blair A, Holmes FF, Boysen CD, Robel RJ, Hoover R, Fraumeni JF Jr.
A population-based case-control study of soft-tissue sarcoma (STS), Hodgkin's disease (HD), and non-Hodgkin's lymphoma (NHL) in Kansas found farm herbicide use to be associated with NHL (odds ratio [OR], 1.6; 95% confidence interval [CI], 0.9, 2.6). Relative risk of NHL increased significantly with number of days of herbicide exposure per year and latency. Men exposed to herbicides more than 20 days per year had a sixfold increased risk of NHL (OR, 6.0; 95% CI, 1.9, 19.5) relative to nonfarmers. Frequent users who mixed or applied the herbicides themselves had an OR of 8.0 (95% CI, 2.3, 27.9) for NHL. Excesses were associated with use of phenoxyacetic acid herbicides, specifically 2,4-dichlorophenoxyacetic acid. Neither STS nor HD was associated with pesticide exposure. This study confirms the reports from Sweden and several US states that NHL is associated with farm herbicide use, especially phenoxyacetic acids. It does not confirm the case-control studies or the cohort studies of pesticide manufacturers and Vietnam veterans linking herbicides to STS or HD.
[THE ERRATUM IS NO MORE THAN A CHANGE IN THE TITLE OF THE MAIN RESULTS TABLE.]

Hardell L, Sandstrom A. 1979 Jun. Case-control study: soft-tissue sarcomas and exposure to phenoxyacetic acids or chlorophenols. Br J Cancer 39:711-7.
Abstract: In 1977 a number of patients with soft-tissue sarcomas and previous exposure to phenoxyacetic acids were described. Following from these observations a matched case-control study was made. Exposure to chlorophenols was also included in this study. The results showed that exposure to phenoxyacetic acids or chlorophenols gave an approximately 6-fold increase in the risk for this type of tumour. It was not possible to determine, however, whether the carcinogenic effect was exerted by these compounds or by impurities such as chlorinated dibenzodioxins and dibenzofurans that in almost all cases were part of the commercial preparations.

J Natl Cancer Inst. 1990 Mar 21;82(6):486-90. Comment in: * J Natl Cancer Inst. 1990 Nov 21;82(22):1785-6.
Exposure to dioxins as a risk factor for soft tissue sarcoma: a population-based case-control study. Eriksson M, Hardell L, Adami HO. Department of Oncology, University Hospital, Umea, Sweden.
In a case-control study including 237 cases with soft tissue sarcoma and 237 controls, previous jobs and exposures to different agents, including pesticides, were assessed. Exposure to phenoxyacetic acids or chlorophenols gave a statistically significant increased rate ratio (RR) of 1.80 [95% confidence interval (CI) = 1.02-3.18] for soft tissue sarcoma. Exposure to phenoxyacetic acids of all types gave a nonsignificantly increased RR of 1.34 (95% CI = 0.70-2.56). During the 1950s, exposure to 2,4,5-trichlorophenoxyacetic acid gave a threefold significantly increased risk. High-grade exposure to chlorophenols, which are also contaminated by dioxins, gave an RR of 5.25 (95% CI = 1.69-16.34). The increased risk was thus attributed to dioxin-contaminated phenoxyacetic acids or chlorophenols that gave an RR of 2.43 (95% CI = 1.30-4.54). PMID: 2313720 [PubMed - indexed for MEDLINE]

Cancer. 1988 Aug 1;62(3):652-6. The association between soft tissue sarcomas and exposure to phenoxyacetic acids. A new case-referent study. Hardell L, Eriksson M. Department of Oncology, University Hospital, Umea, Sweden.
A case-referent study on soft tissue sarcomas (STS) was conducted, to see if previous findings regarding an association between exposure to phenoxyacetic acids or chlorophenols and this tumor type could be reproduced. Fifty-five male STS patients were thereby compared with 220 living and 110 dead population-based referents. Furthermore, another referent group consisting of 190 patients with another type of malignant disease was used in order to evaluate any influence of

recall bias on the results. To obtain information about exposure to the studied chemicals, as well as about any other exposures that might be of interest, questionnaires were used, and if necessary these were completed over the phone by an interviewer who had no information regarding case-referent status. All analysis and interpretation of exposure data were done in a blinded manner. Exposure to phenoxyacetic acids gave a roughly three-fold increased risk for STS, thereby confirming previous findings, whereas exposure to chlorophenols was not associated with STS in this study. PMID: 3390800 [PubMed - indexed for MEDLINE]

Lakartidningen. 1981 Aug 19;78(34):2862-3. [Phenoxyacetic acid, chlorphenols and cancer] [Article in Swedish] Hardell L, Eriksson M. PMID: 7321672 [PubMed - indexed for MEDLINE]

Br J Cancer. 1981 Feb;43(2):169-76. Malignant lymphoma and exposure to chemicals, especially organic solvents, chlorophenols and phenoxy acids: a case-control study. Hardell L, Eriksson M, Lenner P, Lundgren E.
A number of men with malignant lymphoma of the histiocytic type and previous exposure to phenoxy acids or chlorophenols were observed and reported in 1979. A matched case-control study has therefore been performed with cases of malignant lymphoma (Hodgkin's disease and non-Hodgkin lymphoma). This study included 169 cases and 338 controls. The results indicate that exposure to phenoxy acids, chlorophenols, and organic solvents may be a causative factor in malignant lymphoma. Combined exposure of these chemicals seemed to increase the risk. Exposure to various other agents was not obviously different in cases and in controls. PMID: 7470379 [PubMed - indexed for MEDLINE]

Lakartidningen. 1979 Oct 31;76(44):3872-5. [Case- control study of malignant mesenchymal soft tissue tumors and exposure to chemical substances] [Article in Swedish] Eriksson M, Hardell L, Berg NO, Moller T, Axelson O. PMID: 529930 [PubMed - indexed for MEDLINE]

Kogevinas M, Kauppinen T, Winkelmann R, Becher H, Bertazzi PA, Buenodemesquita HB, Coggon D, Green L, Johnson E, Littorin M, Lynge E, Marlow DA, Mathews JD, Neuberger M, Benn T, Pannett B, Pearce N, Saracci R. 1995. Soft tissue sarcoma and non-hodgkins lymphoma in workers exposed to phenoxy herbicides, chlorophenols, and dioxins - TWO nested case-control studies. Epidemiology 6:396-402.
Abstract: We examined the effect of exposure to chemicals present in the production and spraying of phenoxy herbicides or chlorophenols in two nested case-control studies of soft tissue sarcoma and non-Hodgkin's lymphoma. Eleven sarcoma and 32 lymphoma cases occurring within an international cohere were matched for age, sex, and country of residence with 55 and 158 controls, respectively. Exposures to 21 chemicals or mixtures were estimated by three industrial hygienists who were blind to the subject's case-control status. Excess risk of soft tissue sarcoma was associated with exposure to any phenoxy herbicide [odds ratio (OR) = 10.3; 95% confidence interval (CI) 1.2-91] and to each of the three major classes of phenoxy herbicides (2,4-dichlorophenoxyacetic acid, 2,4,5-trichlorophenoxyacetic acid, and 4-chloro-2-methylphenoxyacetic acid), to any polychlorinated dibenzodioxin or furan (OR = 5.6; 95% CI = 1.1-28), and to 2,3,7,8-tetrachlorodibenzo-p-dioxin (OR = 5.2; 95% CI = 0.85-32). Sarcoma risk was not associated with exposure to raw materials or other process chemicals. In the non-Hodgkin's lymphoma study, associations were generally weaker than those found in the study on sarcoma. These findings indicate that workers exposed to phenoxy herbicides and their contaminants are at a higher risk of soft tissue sarcoma. Keywords:

Lancet. 1991 Oct 26;338(8774):1027-32. Comment in: * Lancet. 1991 Nov 30;338(8779):1392-3.
Cancer mortality in workers exposed to chlorophenoxy herbicides and chlorophenols.
Saracci R, Kogevinas M, Bertazzi PA, Bueno de Mesquita BH, Coggon D, Green LM, Kauppinen T, L'Abbe KA, Littorin M, Lynge E, et al. Unit of Analytical Epidemiology, International Agency for Research on Cancer, Lyon, France.
Epidemiological studies have revealed an increased risk of cancer, notably soft-tissue sarcomas and non-Hodgkin's lymphomas, in people occupationally exposed to chlorophenoxy herbicides, including those contaminated by 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD). We report here a historical cohort study of mortality in an international register of 18,910 production workers or sprayers from ten countries. Exposure was reconstructed through questionnaires, factory or spraying records, and job histories. Cause-specific national death rates were used as reference. No excess was observed in all-cause mortality, for all neoplasms, for the most common epithelial cancers, or for lymphomas. A statistically non-significant two-fold excess risk, based on 4 observed deaths, was noted for soft-tissue sarcoma with a standardised mortality ratio (SMR) of 196 and 95% confidence interval (CI) 53-502; this was concentrated as a six-fold statistically significant excess, occurring 10-19 years from first exposure in the cohort as a whole (SMR = 606 [165-1552]) and, for the same time period, as a nine-fold excess among sprayers (SMR = 882 [182-2579]). Risks appeared to be increased for cancers of the testicle, thyroid, other endocrine glands, and nose and nasal cavity, based on small numbers of deaths. The excess of soft-tissue sarcomas among sprayers is compatible with a causal role of chlorophenoxy herbicides but the excess does not seem to be specifically associated with those herbicides probably contaminated by TCDD.
Publication Types: * Clinical Trial * Multicenter Study PMID:1681353 [PubMed - indexed for MEDLINE]
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NEGATIVE RESULTS/GENERAL

We believe that many of these authors have enormous financial ties to the 2,4-D industry, irrespective of what journal they have managed to get their studies published in. For example Gavazza, lead investigator of most of the negative CML findings below, was hired by the 2,4-D Industry Task Force to investigate the positive CML findings that had undergone high quality peer review. Other than these questionably-published CML negative results, it is highly notable that there are hardly any negative results published, no matter the quality of the journal.

NEGATIVE RESULTS/NHL AND ASTROCYTOMA

Bond GG, Wetterstroem NH, Roush GJ, McLaren EA, Lipps TE, Cook RR. 1988 Feb. Cause specific mortality among employees engaged in the manufacture, formulation, or packaging of 2,4-dichlorophenoxyacetic acid and related salts. Br J Ind Med 45:98-105.
Abstract: Mortality is reported to the end of 1982 for 878 chemical workers potentially exposed to 2,4-dichlorophenoxyacetic acid (2,4-D) at any time between 1945 and 1983. Observed mortality was compared with expected levels based on adjusted rates for United States white men and for other male employees from this manufacturing location who were not exposed to 2,4-D. Because of a recently reported increased incidence of astrocytomas in male rats fed the highest dose level of 2,4-D, special attention was given to deaths from brain neoplasms in the cohort. None was observed. The absence of an increased risk of brain cancer in people exposed to 2,4-D is supported by studies of other exposed populations and those studies are briefly reviewed. Moreover, in the present study, analyses by production area, duration of exposure, and cumulative dose showed no patterns suggestive of a causal association between 2,4-D exposure and any other particular cause of death.

NEGATIVE RESULTS/NHL

Bloemen LJ, Mandel JS, Bond GG, Pollock AF, Vitek RP, Cook RR. 1993 Dec. An update of mortality among chemical workers potentially exposed to the herbicide 2,4-dichlorophenoxyacetic acid and its derivatives. J Occup Med 35:1208-12.
Abstract: Four years of additional mortality follow-up through 1986 are reported for a previously studied cohort of 878 chemical workers who were potentially exposed to 2,4-dichlorophenoxyacetic acid (2,4-D) and its derivatives between 1945 and 1983. Observed mortality was compared with expected levels based on death rates of the US population and of 36,804 "unexposed" workers from the same manufacturing location. Non-Hodgkin's lymphoma (NHL) was a particular focus of the study because of a suggested association with 2,4-D exposure in some case-control studies. For the total observation period, the standardized mortality ratios for all causes and for malignant neoplasms were 92 and 91, respectively. Analyses using the internal comparison group yielded virtually identical results. The initial study had found two deaths from NHL, both of which occurred under circumstances (ie, short latency and modest exposure) which made it less plausible that they were related to 2,4-D exposure. No new deaths from NHL were observed in the extended follow-up period and mortality for this cause showed a nonstatistically significant excess (standardized mortality ratio, 196; 95% confidence interval 24 to 708) for the total observation period. Analyses by production area, and by two different measures of exposure, combined with two different approaches to account for latency, did not show patterns suggestive of a causal relationship between exposure to 2,4-D or its derivatives and any particular cause of death.Keywords:

Burns CJ, Beard KK, Cartmill JB. 2001 Jan. Mortality in chemical workers potentially exposed to 2,4-dichlorophenoxyacetic acid (2,4-d) 1945-94: an update. Occupational & Environmental Medicine 58:24-30.
Abstract: Objective-To update and add to a previously identified cohort of employees potentially exposed to the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D). The putative association between 2,4-D and non-Hodgkin's lymphoma has been debated for more than a decade. Methods-Cohort members were male employees of The Dow Chemical Company who manufactured or formulated 2,4-D any time from 1945 to the end of 1994. Their mortality experience was compared with national rates and with more than 40 000 other company employees who worked at the same location. Results-330 Deaths were observed among 1517 people compared with 365 expected (standardised mortality ratio (SMR)=0.90, 95% confidence interval (95% CI) 0.81 to 1.01). There were no significantly increased SMRs for any of the causes of death analyzed. When compared with the United States rates, the SMR for non-Hodgkin's lymphoma (NHL) was 1.00 (95% CI 0.21 to 2.92). The internal comparison with other Dow employees showed a non-significant relative risk of 2.63, (95% CI 0.85 to 8.33). Death was attributed to amyotrophic lateral sclerosis (ALS) for three cohort members. Compared with the other company employees, the relative risk was 3.45 (95% CI 1.10 to 11.11). The cases were employed in the manufacture or formulation of 2,4-D at different periods (1947-9, 1950-1, and 1968-86), and for varying durations of time (1.3, 1.8, and 12.5 years). Conclusion-There was no evidence of a causal association between exposure to 2,4-D and mortality due to all causes and total malignant neoplasms. No significant risk due to NHL was found. Although not an initial hypothesis, an increased relative risk of ALS was noted. This finding is unsupported by other animal and human studies. [References: 48] Number of References 48

Keywords:

Wiklund K, Holm LE. 1986 Feb. Soft tissue sarcoma risk in Swedish agricultural and forestry workers. J Natl Cancer Inst 76:229-34.
Abstract: The risk of soft tissue sarcoma following possible exposure to phenoxy acid herbicides was studied in 354,620 Swedish men, who were employed in agriculture or forestry according to a national census in 1960. This cohort was further divided into six subcohorts, on assumed exposure to phenoxy acid herbicides. The most commonly used phenoxy acid in Sweden was (4-chloro-2-methylphenoxy)acetic acid (CAS: 94-74-6). The reference cohort encompassed 1,725,845 Swedish men employed in other industries. All persons were followed up in the cancer-environment register during the period 1961-79. A total of 331 cases of soft tissue sarcomas was observed in the study cohort and there were 1,508 cases in the reference group [relative risk (RR), 0.9; 95% confidence interval, 0.8-1.0]. No subcohort of agricultural or forestry workers showed any significantly increased RR, nor was there any significant difference in RR between the subcohorts. Despite the greatly increased use of phenoxy acid herbicides from 1947 to 1970, no time-related increase in the RR of soft tissue sarcoma was found in the total cohort or in any of the subcohorts.

NEGATIVE RESULTS/NHL ANALOGUE: CANINE MALIGNANT LYMPHOMA (CML)

Edwards MD, Pazzi KA, Gumerlock PH, Madewell BR. 1993. C-n-ras is activated infrequently in canine malignant lymphoma. Toxicol Pathol 21:288-291.
Abstract: Activated c-N-ras alleles have been detected in human lymphoma specimens. The aim of the present study was to determine the frequency of c-N-ras mutational activation in canine malignant lymphoma. DNA was isolated from 28 canine malignant lymphoma specimens collected from 28 separate dogs and examined for c-N-ras mutations by polymerase chain reaction amplification and direct sequencing. The tumors were naturally occurring and derived from 20 dogs with known exposures to the phenoxy herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) and from 8 dogs with no known exposure to the herbicide. An oncogenically activating mutation was found in 1 dog without known 2,4-D exposure. The mutation was a 13th codon, second position transition that would result in a glycine-to-aspartate amino acid substitution. The results of this study demonstrate that, similar to the human, c-N-ras mutations are uncommon in dogs with malignant lymphoma and that there is no association between 2,4-D exposure and activation of c-N-ras in the dog.
Keywords:

Gavazza A, Presciuttini S, Barale R, Lubas G, Gugliucci B. 2001 May-2001 Jun 30. Association between canine malignant lymphoma, living in industrial areas, and use of chemicals by dog owners. J Vet Intern Med 15:190-195.
Abstract: A case-control study was carried out to determine whether residential exposure to environmental pollutants increased risk for canine lymphoma in pet dugs. One hundred one cases with cyctologically or histologically confirmed lymphoma diagnosed at a veterinary teaching hospital between the middle of 1996 and the middle of 1998 were examined. Controls were obtained by choosing twice the number of dogs without neoplastic disease. with overlapping distributions of province of residence, age, sex. and breed. Information regarding animal management. residence type, professional or hobby use of chemicals by owners, and treatment with herbicides or other pesticides in the area li frequently visited by the dogs was obtained with a multiple-choice questionnaire by telephone interview. Two variables were positively and independently associated with the disease. namely residency in industrial areas (odds ratio [OR]: = 8.5: 95% confidence interval [CI]. 2.3-30.9) and use of chemicals by owners, specifically paints or solvents (OR = 4.6: 95% CI. 1.7-12.6). A significantly lower value of the mean apr of disease onset was found in the group of dogs at risk in comparison with the group of all other dogs (6.1 +/- 0.4 years, n = 36 versus 7.5 +/- 0.4 years. n = 65, respectively; P = .008). Variables describing animal care and pesticide use were either not associated with the disease or were uninformative. We suggest that canine lymphoma may be considered a sentinel of potentially hazardous situations for humans, because of the relatively short latency between exposure and disease onset. [References: 27] Number of References 27
Keywords:

Kaneene JB, Miller R. 1999 Jun. Re-analysis of 2,4-d use and the occurrence of canine malignant lymphoma. Veterinary & Human Toxicology 41:164-170.
Abstract: An independent scientific review panel had concerns involving study design, analysis and interpretation of results in a case-control study investigating the relationship between canine malignant lymphoma(CML) and the use of 2,4-D herbicide. To address these cone-ems, a re-analysis was done to examine 2,4-D use and its association with CML. This case-control study re-analyzed the data using the exposure definition used in the original study, re-analyzed the data using a redefinition of exposure, and conducted a dose-response analysis with the redefined exposure criteria. Our results agreed with the original author's analyses that no effects were found when stratifying by survey method and geographic region, and that there. were no significant differences between separated and pooled control groups. However, we did not confirm a dose-response relationship between 2,4-D use and CML. Additionally, the occurrence of CML was not found significantly associated with the use of 2,4-D. [References: 4] Number of References 4
Keywords:

O'Brien DJ, Kaneene JB, Getis A, Lloyd JW, Swanson GM, Leader RW. 2000 Nov 16. Spatial and temporal comparison of selected cancers in dogs and humans, michigan, usa, 1964-1994. Prev Vet Med 47:187-204.
Abstract: Our aim was to investigate the geographic acid time distributions of some biologically similar neoplasms in dogs and humans living in Michigan, USA, between 1964 and 1994. Our objective was to describe and compare the patterns of cancer in the two species while assessing the strength and dependence of those patterns. In this retrospective, registry-based study, histologically confirmed incident human and canine cancer cases were mapped, and second-order (K function) spatial analysis and one-dimensional nearest neighbor temporal analysis were performed on residence addresses and dares of hospital discharge/diagnosis. Included in the study were all 528 incident cases of canine lymphosarcoma, mammary adenocarcinoma, melanoma and spindle-cell sarcomas diagnosed at a veterinary teaching hospital between 1964 and 1994 having residence addresses in Ingham, Oakland, and Wayne Counties; and a stratified random sample of 913 incident human cases of comparable cancers diagnosed during the same time period from the same counties. Results suggest that processes determining spatial aggregation of cases in dogs and humans were not independent of each other, did not act uniformly over different geographic areas, operated at spatial scales <2000 m regardless of species, and tend to act upon dogs more strongly at shorter distances than on humans. Little evidence of interspecies concurrence of temporal clustering was found. (C) 2000 Elsevier Science B.V. All rights reserved.
[References: 57] Number of References 57
Keywords:
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MECHANISMS OF CANCER: MUTAGENICITY (i.e. DNA/CHROMOSONE DAMAGE)

(Most apoptosis/cell-cycle disruption papers are listed in 'cancer/other mechanisms', below. Of course, mutagenicity and cell-cycle (cell replication) disruptions lead to more diseases than just cancer, but cancer is a major endpoint of such damage. The later especially leads to cancer, as both cancer and some cell-cycle disruption involve uncontrolled cell replication.

The weight of the evidence in this subset is notably in opposition to your conclusion. We found just three of the 29 published 2,4-D mutagenicity results gave negative results (i.e. 26 found it mutagenic). Considering the quality negative published findings are at most two, as the third one (published as three sequential papers) is authored by consultants dependent financially on 2,4-D industry, in a journal that accepts authors with horrible financial conflicts of interests.

Given also that the weight ofother evidence clearly places it in EPA's 'possible human carcinogen (Class C)' category, it is important that you consider one of your own (EPA) scientists was one author in a recent study finding that 13 of 13 carcinogens acting through mutagenic mechanisms were on average five to 60 times more potent as carcinogens when the pups were dosed before weaning than when the animals were exposed as adults; at all test doses and in every test (D. Hattis et al. Aug. 2004 'Age-Related Differences in Susceptibility to Carcinogenesis: a quantitative analysis..' Env. Health Perspect.:112:11:1152-8). Obviously, neither the FQPA's 10-fold allowance for child sensitivity, nor your cancer guideline's 10-fold allowance for infants, are sufficient protection, at least for mutagenic carcinogens; yet in this RA you recklessly have decided that children need no such protection from the mutagenic carcinogen 2,4-D. This study and the two studies above showing that 2,4-D is a carcinogen if the animals are dosed early in life (which you failed to test for) all emphasize how poorly all your RAs, including this one, protect children; even though children's exposure to 2,4-D is ubiquitous.

POSITIVE RESULTS/MUTAGENIC

Arias E. 2003 Jul. Sister chromatid exchange induction by the herbicide 2,4-dichlorophenoxyacetic acid in chick embryos. Ecotoxicology & Environmental Safety 55:338-343.
Abstract: As genetic damage may result from exposure to agricultural chemicals, it seemed appropriate to assess the genotoxic potential of 2,4-dichlorophenoxyacetic acid (2,4-D), a widely used broad-leaf herbicide, using a test system that may provide some indications on the genetic risk to animal species in the wild. In the present study, sister chromatid exchange (SCE) induction and cell cycle kinetics alterations by 2,4-D in 4-day old chick embryos were evaluated. Both a commercial herbicide formulation containing 37% 2,4-D isooctyl ester as active ingredient and pure 2,4-D were tested. Chick embryos were treated with 0, 0.5, 1, 2, or 4 mg 2,4-D. Test solutions were applied to the inner shell membrane on day 0 of incubation. Either commercial formulation or pure 2,4-D induced a dose-related increase in SCE frequency over the concentration range from 0 to 4 mg/embryo. Significantly higher SCE frequency was seen for the 4-mg group of embryos treated with the commercial product. A slightly higher SCE value was observed for the vehicle group (acetone-

treated embryos) compared with the negative controls (untreated embryos). Significant inhibition of cell cycle progression was evident in both experimental groups and was generally dose related. The extent of changes in cell kinetics was similar in both groups, although somewhat more marked in the group treated with pure 2,4-D. The present findings corroborate the positive results from recent in vivo rodent studies. (C) 2003 Elsevier Science (USA). All rights reserved. [References: 36] Number of References 36
Keywords:

Ateeq B, Farah MA, Ali MN, Ahmad W. 2002 Feb 15. Clastogenicity of pentachlorophenol, 2,4-d and butachlor evaluated by allium root tip test. Mutation Research-Genetic Toxicology & Environmental Mutagenesis 514:105-113.
Abstract: The meristematic mitotic cells of Allium cepa is an efficient cytogenetic material for chromosome aberration assay on environmental pollutants. For assessing genotoxicity of pentachlorophenol (PCP), 2,4-dichlorophenoxyacetic acid (2,4-D) and 2-chloro-2,6-diethyl-N-(butoxymethyl) acetanilide (butachlor), 50% effective concentration (EC50), c-mitosis, stickiness, chromosome breaks and mitotic index (MI) were used as endpoints of genotoxicity. EC50 values for PCP and butachlor are 0.73 and 5.13 ppm, respectively. 2,4-D evidently induced morphological changes at higher concentrations. Some changes like crochet hooks, c-tumours and broken roots were unique to 2,4-D at 5-20 ppm. No such abnormalities were found in PCP and butachlor treated groups, however, root deteriorated and degenerated at higher concentrations (<3 ppm) in PCP. MI in 2,4-D showed a low average of 14.32% followed by PCP (19.53%), while in butachlor it was recorded 71.6%, which is near to the control value. All chemicals induced chromosome aberrations at statistically significant level. The highest chromosome aberration frequency (11.90%) was recorded in PCP at 3 ppm. Large number of c-mitotic anaphases indicated that butachlor acts as potent spindle inhibitor, whereas, breaks, bridges, stickiness and laggards were most frequently found in PCP showing that it is a potent clastogen. (C) 2002 Elsevier Science B.V. All rights reserved. [References: 30] Number of References 30 Keywords:

Ateeq B, Abul farah M, Niamat Ali M, Ahmad W . 2002 Jul 25. Induction of micronuclei and erythrocyte alterations in the catfish Clarias batrachus by 2,4-dichlorophenoxyacetic acid and butachlor. Mutat Res 518:135-44.
Abstract: The micronucleus test (MNT) in fish erythrocytes has increasingly been used to detect the genotoxic effects of environmental mutagens and its frequency is considered to reflect the genotoxic damage to cells, mainly the chromosomes. Besides, morphologically altered erythrocyte is taken as an index of cytotoxicity. Both parameters were used in the present study by two herbicides, 2,4-dichlorophenoxyacetic acid (2,4-D, in 25, 50 and 75ppm concentrations) and 2-chloro-2,6-diethyl-N-(butoxymethyl) acetanilide (butachlor, in 1, 2 and 2.5ppm concentrations) for genotoxic and cytotoxic endpoints. The study was carried out by an in vivo method on peripheral erythrocytes of catfish Clarias batrachus using multiple sampling times (48, 72 and 96h). Cytogenetic preparations were made by haematoxylin-eosin staining technique. Pycnotic and granular micronuclei (MN) were consistently observed irrespective of chemical tested. A wide range of altered cells was also observed. Echinocytes accompanied by altered nuclei and vacuoles were prominent feature of 2,4-D, whereas, anisochromasia and anisocytosis of erythrocytes were characteristic of butachlor. Increase in MN as well as altered cells frequencies were significant. A positive dose-response relationship in all exposures and sampling times was observed. Herbicides used were found to be genotoxic as well as cytotoxic in this fish. The suitability of the adopted parameters for the screening of the aquatic genotoxicants is discussed.

Figgs LW, Holland NT, Rothmann N, Zahm SH, Tarone RE, Hill R, Vogt RF, Smith MT, Boysen CD, Holmes FF, VanDyck K, Blair A. 2000 Apr. Increased lymphocyte replicative index following 2,4-dichlorophenoxyacetic acid herbicide exposure. Cancer Causes Control 11:373-80.
Abstract: OBJECTIVE: Evaluate peripheral blood lymphocyte proliferation (replicative index:RI) and micronuclei frequency (MF) among 2,4-D herbicide applicators. METHODS: Twelve applicators spraying only 2,4-D provided a blood and urine specimen upon enrollment, several urine samples during the spraying season, and a blood specimen at the study's end. Nine controls provided blood and urine specimens upon enrollment and at the study's end. Gas chromatography/tandem mass spectroscopy determined urinary 2,4-D levels and standard in-vitro assays determined RI and MF scores. Applicator RI and MF were compared before and after spraying and with controls. RESULTS: Applicators contributed 45 urine specimens with concentrations ranging from 1.0 to 1700 (microg 2,4-D/g creatinine/L urine) that logarithmically (In) increased as spraying time increased. Applicator RI increased after spraying (p = 0.016), independent of tobacco and alcohol use, and demonstrated a weak dose-response with increasing urinary 2,4-D levels (p = 0.15). Among 2,4-D applicators, pre-exposure complete blood counts and lymphocyte immunophenotypes were not significantly different from post-exposure measurements. CONCLUSION: Urinary 2,4-D concentration, an exposure biomarker, may be associated with lymphocyte replicative index, a cell proliferation biomarker.

Holland NT, Duramad P, Rothman N, Figgs LW, Blair A, Hubbard A, Smith MT. 2002 Nov 26. Micronucleus frequency and proliferation in human lymphocytes after exposure to herbicide 2,4-dichlorophenoxyacetic acid in vitro and in vivo. Mutation Research-Genetic Toxicology & Environmental Mutagenesis 521:165-178.
Abstract: Widespread use of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) and its association with non-Hodgkin's lymphoma (NHL) and other cancers has raised public concern. Here, micronucleus (MN) formation has been used as a

biomarker of genotoxicity, and replicative and mitotic indices (MIs) as biomarkers of cell cycle kinetics in human lymphocytes. Cells were cultured either as whole blood or isolated lymphocytes and treated with pure or commercial forms of 2,4-D at doses between 0.001 and 1 mM for 48 h. Exposure to 2,4-D produced a minimal increase in MN in whole blood and even smaller one in isolated lymphocyte cultures. This induction took place only at levels approaching cytotoxicity and was accompanied by a significant inhibition of replicative index (RI). At a low (0.005 mM) dose of commercial 2,4-D, a small, marginally significant increase in RI (12-15%) was found in two independent sets of experiments (P = 0.052). Additionally, we found that lymphocyte RI was more affected by commercial 2,4-D containing 9.4% of the chemically pure 2,4-D, than with an equal concentration of the latter suggesting that other ingredients present in the commercial pesticide may be responsible or may enhance the effect of 2,4-D. Mitotic index, however, did not show any significant change with either commercial or pure 2,4-D. The lymphocytes of 12 male applicators exposed solely to 2,4-D during a 3-month period had a significantly higher RI than the same group prior to exposure and than a control group (P < 0.01), in accordance with the in vitro finding of increased RI at low doses. (C) 2002 Elsevier Science B.V. All rights reserved. [References: 61] Number of References 61Keywords: **[NOTE THE LOW (5 NANOMOLE) DOSE EFFECT]**

Kaya B, Yanikoglu A, Marcos R. 1999. Genotoxicity studies on the phenoxyacetates 2,4-d and 4-cpa in the drosophila wing spot test. Teratogenesis, Carcinogenesis, & Mutagenesis 19:305-312.
Abstract: The phenoxyacetates 2,4-D and 4-CPA were evaluated for genotoxicity using the Drosophila melanogaster wing spot test, which assesses for somatic mutation and recombination events. Third-instar larvae trans-heterozygous for two recessive mutations affecting the expression of wing trichomes, multiple wing hairs (mwh), and flare (flr) were treated by chronic feeding with different concentrations of the two chemicals. Feeding lasted until pupation of the surviving larvae and the genotoxic effects induced were evaluated in adults for the appearance of wing-blade cell clones with the mwh, flr or mwh-flr phenotypes. Exposure to 2,4-D, at the highest concentration evaluated (10 mM), induced a weak but significant increase in the frequency of two of the categories of recorded spots: large single and total spots; in contrast, the 4-CPA treatments failed to induce any significant increase in the frequency of evaluated spots. When the heterozygous larvae for mwh and the multiple inverted TM3 balancer chromosome were treated with the chemicals, no increases were detected, either after the 2,4-D nor the 4-CPA treatments. (C) 1999 Wiley-Liss, Inc. [References: 30] Number of References 30
Keywords:

Kornuta N, Bagley E, Nedopitanskaya N. 1996. Genotoxic effects of pesticides. J Environ Pathol Toxicol Oncol 15: 75-8.
Abstract: Epidemiologic data showed an increase in the number of cancer cases in persons involved in agricultural production using pesticides. According to IARC, more than 25% of pesticides are classified as oncogens. In recent years, the concept of malignant tumors developing after environmental contamination with chemicals has been accepted. Changes in genetic material are at the basis of this process because many environmental pollutants are chemical carcinogens and mutagens with the capacity of causing DNA damage. DNA damage was proposed as a useful parameter for assessing the genotoxic properties of environmental pollutants. The correlation between exposure to carcinogenic substance and the level of DNA damage is essential. Pesticides are highly biologically active chemicals. They may interact with DNA and damage its structure. Such interaction may be critical for the manifestation of carcinogenic properties of different chemicals. We report on the organotropic genotoxic effects of different chemical classes of pesticides (decis, cypermetrin, 2,4-D, polyram) studied by means of alkaline unwinding assay DNA.

Filkowski J, Besplug J, Burke P, Kovalchuk I, Kovalchuk O. 2003 Dec 9. Genotoxicity of 2,4-d and dicamba revealed by transgenic arabidopsis thaliana plants harboring recombination and point mutation markers . Mutation Research-Genetic Toxicology & Environmental Mutagenesis 542:23-32.
Abstract: The phenoxy herbicides 2,4-D and dicamba are released daily into the environment in large amount. The mechanisms of genotoxicity and mutagenicity of these herbicides are poorly understood, and the available genotoxicity data is controversial. There is a cogent need for a novel genotoxicity monitoring system that could provide both reliable information at the molecular level, and complement existing systems. We employed the transgenic Arabidopsis thaliana 'point mutation' and 'recombination' plants to monitor the genetic effects of the herbicides 2,4-D and dicamba. We found that both herbicides had a significant effect on the frequency of homologous recombination A --> G mutation. Neither herbicides affected the T --> G mutation frequency. Interestingly, these transgenic biomonitoring plants were able to detect the presence of phenoxy herbicides at concentrations that were lower than the guideline levels for Drinking Water Quality. The results of our studies suggest that our transgenic system may be ideal for the evaluation of the genotoxicity of herbicide-contaminated water. Moreover, the unique ability of the plants to detect both double-strand breaks (homologous recombination) and point mutations provides tremendous potential in the study of molecular mechanisms of genotoxicity and mutagenicity of phenoxy herbicides. (C) 2003 Elsevier B.V. All rights reserved. [References: 40] Number of References 40
Keywords:

Garry VF, Tarone RE, Kirsch IR, Abdallah JM, Lombardi DP, Long LK, Burroughs BL, Barr DB, Kesner JS. 2001 May. Biomarker correlations of urinary 2,4-d levels in foresters: genomic instability and endocrine disruption. Environ Health Perspect 109:495-500.

Abstract: Forest pesticide applicators constitute a unique pesticide use group. Aerial, mechanical-ground, and focal weed control by application of herbicides, in particular chlorophenoxy herbicides, yield diverse exposure scenarios. In the present work, we analyzed aberrations in G-banded chromosomes, reproductive hormone levels, and polymerase chain reaction-based V(D)J rearrangement frequencies in applicators whose exposures were mostly limited to chlorophenoxy herbicides. Data from appliers where chlorophenoxy use was less frequent were also examined. The biomarker outcome data were compared to urinary levels of 2,4-dichlorophenoxyacetic acid (2,4-D) obtained at the time of maximum 2,4-D use. Further comparisons of outcome data were made to the total volume of herbicides applied during the entire pesticide-use season. Twenty-four applicators and 15 minimally exposed foresters (control) subjects were studied. Categorized by applicator method, men who used a hand-held, backpack sprayer in their applications showed the highest average level (453.6 ppb) of 2,4-D in urine. Serum luteinizing hormone (LH) values were correlated with urinary 2,4-D levels, but follicle-stimulating hormone and free and total testosterone were not. At the height of the application season; 6/7 backpack sprayers, 3/4 applicators who used multinozzle mechanical (boom) sprayers, 4/8 aerial applicators, and 2/5 skidder-radiarc (closed cab) appliers had two or more V(D)J region rearrangements per microgram of DNA. Only 5 of 15 minimally exposed (control) foresters had two or more rearrangements, and 3 of these 5 subjects demonstrated detectable levels of 2,4-D in the urine. Only 8/24 DNA samples obtained from the exposed group 10 months or more after their last chlorophenoxy use had two rearrangements per microgram of DNA, suggesting that the exposure-related effects observed were reversible and temporary. Although urinary 2,4-D levels were not correlated with chromosome aberration frequency, chromosome aberration frequencies were correlated with the total volume of herbicides applied, including products other than 2,4-D. In summary, herbicide applicators with high urinary levels of 2,4-D (backpack and boom spray applications) exhibited elevated LH levels. They also exhibited altered genomic stability as measured by V(D)J rearrangement frequency, which appears reversible months after peak exposure. Though highly detailed, the limited sample size warrants cautious interpretation of the data. [References: 28] Number of References 28
Keywords:

K. Atanassov 1992 ‘Effect of the herbicide Schpritshormit’ (salt in 2,4-D) Animal Science 29:54-61. [When administered in rabbits’ drinking water, the sodium salt of 2,4-D caused an increase in the number of chromosomes, brain cells with too many chromosomes and cells with multiple chromosome sets.]
[ALSO LISTED IN 'POSITIVE RESULTS/BRAIN CANCERS' ABOVE]

The dimethyl amine salt of 2,4-D caused breaks in DNA molecules (genetic material) from human connective tissue. M. Clausen et al. 1990 ‘Comparison of the cytotoxicity and DNA-damaging properties of 2,4-D’ Arch. Toxicol. 64:497-501.

Turkula TE, Jalal SM. 1985 May-Jun. Increased rates of sister chromatid exchanges induced by the herbicide 2,4-D. J Hered 76:213-4.
Abstract: The potential for genetic damage from widely used hormonic herbicides, such as 2,4-dichlorophenoxyacetic acid (2,4-D), continues to be of serious concern. The mutagenic effect as reflected by the rates of sister chromatid exchanges (SCE) was determined in cultured human lymphocytes. Data were based on the analysis of 50 cells for the control and each of the three treatments. A 50 micrograms/ml dosage caused a highly significant increase in SCE. Dosages of 100 and 250 micrograms/ml elevated the rate of SCE, but not significantly. Since 2,4-D biodegrades rapidly in soil and water, its continued use is not in serious question until safer compounds are available. However, the results of this study suggest that the danger of genetic damage from direct exposure to commercial samples of 2,4-D should not be ignored.

Madrigal-Bujaidar E, Hernandez-Ceruelos A, Chamorro G. 2001 Sep. Induction of sister chromatid exchanges by 2,4-dichlorophenoxyacetic acid in somatic and germ cells of mice exposed in vivo. Food & Chemical Toxicology 39:941-946.
Abstract: 2,4-dichlorophenoxyacetic acid (2,4-D) is one of the most widely used selective herbicides throughout the world; however, the studies that have been conducted to establish its genotoxic potential have given conflicting results. The aim of this investigation was to determine whether the herbicide increases the frequency of sister chromatid exchanges (SCEs) in bone marrow and spermatogonial cells of mice exposed in vivo. The experiment included an oral administration of 2,4-D to three groups of mice (50, 100 and 200 mg/kg), as well as to a control group of animals administered with distilled water, pH 10.5 and another group injected with cyclophosphamide (50 mg/kg). In somatic cells, the results showed a significant SCE increase with the two high doses tested, a response that was manifested in a dose-dependent manner. With regard to the mitotic index and the cell proliferation kinetics, there were no modifications exerted by 2,4-D; however, cyclophosphamide induced cytotoxic damage and a cell-cycle delay. With respect to the germ cells, the. genotoxic results were similar to those described earlier; that is, there was a significant SCE increase induced by the two high 2,4-D doses tested and a higher genotoxic damage was observed in the animals treated with cyclophosphamide. Our investigation established that 2,4-D is a moderate genotoxicant in mice treated in vivo with high doses, and suggests a minor hazard for humans in the present conditions of its use. (C) 2001 Elsevier Science Ltd. All rights reserved.
[References: 42] Number of References 42
Keywords:

Korte C, Jalal SM. 1982 May-Jun. 2,4-D induced clastogenicity and elevated rates of sister chromatid exchanges in cultured human lymphocytes. J Hered 73:224-6.

Abstract: Potential for genetic damage in future generations from such widely used hormonic herbicide as 2,4-D (2,4-dichlorophenoxyacetic acid) is of serious concern. Yet the data, particularly on mammalian systems, continue to be inadequate and inconclusive. An attempt was made in this study to determine the clastogenic and mutagenic potential of 2,4-D in cultured lymphocytes. Chromosome damage though statistically insignificant occurred at dosages as low as 0.2 microgram/ml. Chromosome damage was increased at a statistically significant level whenever the concentration was 50 microgram/ml or higher. Mutagenicity, based on rates of increase in sister chromatid exchanges, was significant at 10 micrograms/ml of higher concentrations. Statistical testing was based o analysis of variance, Dunnett's multiple comparison tests and linear regressions. It seems imperative therefore to avoid indiscriminate use of 2,4-D, and to test the compound for long-range low-level exposures.

Arch Toxicol. 1989;63(3):203-8. Effects of commercial chlorophenolate, 2,3,7,8-TCDD, and pure phenoxyacetic acids on hepatic peroxisome proliferation, xenobiotic metabolism and sister chromatid exchange in the rat. Mustonen R, Elovaara E, Zitting A, Linnainmaa K, Vainio H. Institute of Occupational Health, Department of Industrial Hygiene and Toxicology, Helsinki, Finland.
The induction of hepatic peroxisome proliferation and drug metabolizing enzymes and of sister chromatid exchange (SCE) in lymphocytes was studied in male Han/Wistar rats after exposing them for 2 weeks to a commercial chlorophenolate formulation (Ky-5) (100 mg/kg/day), to 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD; 0.05-5 micrograms/kg/wk) and to the pure phenoxyacetic acids, 2,4-dichlorophenoxyacetic acid (2,4-D; 100 mg/kg/day) and 2-chloro-4-methylphenoxyacetic acid (MCPA; 100 mg/kg/day). The chlorophenolate formulation and pure 2,4-D and MCPA caused significant increases in the number of peroxisomes in liver cells, although the average size of peroxisomes was not affected, whereas the effect of even the highest dose of 2,3,7,8-TCDD remained small. This finding indicates that dioxin impurities do not account for the peroxisome proliferation induced by chlorophenolate. The relative weight of the liver increased significantly in rats treated with the chlorophenolate formulation and with 2,3,7,8-TCDD (5.0 and 0.5 micrograms/kg). The pattern of induction of xenobiotic metabolizing enzymes showed some differences between chlorophenolate treatment and 2,3,7,8-TCDD treatment. Furthermore, the effects of pure phenoxyacetic acids were different from that seen with chlorophenolate and 2,3,7,8-TCDD. The highest dose of 2,3,7,8-TCDD increased the frequency of SCE in circulating lymphocytes slightly, but significantly. PMID: 2764706 [PubMed - indexed for MEDLINE]

Mutagenesis. 1986 Jul;1(4):241-5. Effects of phenoxyacetic acids on the induction of chromosome aberrations in vitro and in vivo. Mustonen R, Kangas J, Vuojolahti P, Linnainmaa K. Institute of Occupational Health, Department of Industrial Hygiene and Toxicology, Helsinki, Finland.
The effects of phenoxyacetic acid herbicides were investigated on the induction of chromosome aberrations in human peripheral lymphocyte cultures in vitro and in lymphocytes of exposed workers in vivo. Pure 2,4-dichlorophenoxyacetic acid (2,4-D; 0.125, 0.150, 0.200 and 0.350 mM) did not increase the number of aberrations, whereas the commercial 2,4-D formulation (0.125, 0.250, 0.500, 1.000 and 1.250 mM, with respect to phenoxyacetic acid concentration) significantly increased the number of chromosome aberrations in vitro (without exogenous metabolic activation). The phenoxy acid levels in the breathing zone of the workers varied between 0.3 and 0.4 mg/m3, and the concentrations of phenoxyacetic acids in the urine of the workers after exposure varied from 0.000 to 0.055 mmol/l. There were no increases in chromosome aberrations in peripheral lymphocytes of the exposed subjects. PMID: 3331666 [PubMed - indexed for MEDLINE]

Mustonen R, Kangas J, Vuojolahti P, Linnainmaa K. 1986 Jul. Effects of phenoxyacetic acids on the induction of chromosome aberrations in vitro and in vivo. Mutagenesis 1:241-5.
Abstract: The effects of phenoxyacetic acid herbicides were investigated on the induction of chromosome aberrations in human peripheral lymphocyte cultures in vitro and in lymphocytes of exposed workers in vivo. Pure 2,4-dichlorophenoxyacetic acid (2,4-D; 0.125, 0.150, 0.200 and 0.350 mM) did not increase the number of aberrations, whereas the commercial 2,4-D formulation (0.125, 0.250, 0.500, 1.000 and 1.250 mM, with respect to phenoxyacetic acid concentration) significantly increased the number of chromosome aberrations in vitro (without exogenous metabolic activation). The phenoxy acid levels in the breathing zone of the workers varied between 0.3 and 0.4 mg/m3, and the concentrations of phenoxyacetic acids in the urine of the workers after exposure varied from 0.000 to 0.055 mmol/l. There were no increases in chromosome aberrations in peripheral lymphocytes of the exposed subjects.

Riabchenko NI, Fesenko EV, Antoshchina MM. 1995 Sep-Oct. [A cytogenetic analysis of the combined action of pesticides and irradiation on human lymphocytes]. Radiats Biol Radioecol 35:736-9.
Abstract: The efficiency of the combined action of pesticides and irradiation at the G(o) stage was studied in cultured human lymphocytes. Carbophos (malathion) increased the yield of chromosome and chromatid fragments in irradiated lymphocytes. Herbicide 2,4-D (dichlorophenoxyacetic acid) raised lymphocyte radiosensitivity by increasing the yield of chromosome type aberrations; the radiosensitizing effect of the herbicide decreased as its concentration increased.

Venkov P, Topashka-Ancheva M, Georgieva M, Alexieva V, Karanov E. 2000 Nov. Genotoxic effect of substituted phenoxyacetic acids. Arch Toxicol 74:560-566.
Abstract: The potential toxic and mutagenic action of 2,4-dichlorophenoxyacetic acid has been studied in different test

systems, and the obtained results range from increased. chromosomal damage to no effect at all. We reexamined the effect of this herbicide by simultaneous using three tests based on yeast, transformed hematopoietic, and mouse bone marrow cells. The results obtained demonstrated that 2,4-dichlorophenoxyacetic acid: has cytotoxic and mutagenic effects. The positive response of yeast and transformed hematopoietic cells was verified in kinetics and dose-response experiments. The analysis of metaphase chromosomes indicated a statistically proved induction of breaks, deletions, and exchanges after the intraperitoneal administration of 2,4-dichlorophenoxyacetic acid in mice. The study of phenoxyacetic acid and its differently chlorinated derivatives showed that cytotoxicity and mutagenicity are induced by chlorine atoms at position 2 and/or 4 in the benzene ring. The mutagenic effect was abolished by introduction of a third chlorine atom at position 5. Thus 2,4,5-trichlorophenoxyacetic acid was found to have very weak, if any mutagenic effect; however, the herbicide preserved its toxic effect. [References: 25] Number of References 25 Keywords:

Zeljezic D, Garaj-Vrhovac V. 2004 Jul 15. Chromosomal aberrations, micronuclei and nuclear buds induced in human lymphocytes by 2,4-dichlorophenoxyacetic acid pesticide formulation. Toxicology 200:39-47.
Abstract: Pesticides of worldwide application are used in agriculture in vast amounts each year, of which herbicides are the most prominent class. Phenoxyacetic herbicides constitute one of the largest groups of herbicides sold in the world. Among them, for many years 2,4-dichlorophenoxyacetic acid (2,4-D) has been the one most used. In this study we used Deherban A, a commercial formulation of 2,4-D to determine its possible genotoxic effect on human lymphocytes in vitro by chromosomal aberration analysis and micronucleus assay including the scoring of nuclear buds. Two different concentrations of pesticide formulation were used so that final concentrations of 2,4-D were 0.4 and 4 microg/ml, both in the presence and in the absence of the liver microsomal fraction as metabolic activator. Both concentrations of pesticide caused an increase in chromatid and chromosome breaks, number of micronuclei and number of nuclear buds. Presence of the S9 mix additionally elevated the number of chromatid breaks and micronuclei in treated lymphocytes.

Pavlica M, Papes D, Nagy B. 1991 Jun. 2,4-Dichlorophenoxyacetic acid causes chromatin and chromosome abnormalities in plant cells and mutation in cultured mammalian cells. Mutat Res 263:77-81.
Abstract: The cytotoxic and mutagenic effects of the synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D) on shallot root tip cells and on V79 Chinese hamster fibroblast cells were examined and compared. In shallot root tips 2,4-D caused changes in mitotic activity, as well as changes in chromosome and chromatin structure, and also changes during the cell cycle. 2,4-D also showed mutagenic and cytotoxic effects on V79 cells in culture in concentrations higher than 10 micrograms/ml. The results in both systems (plant and mammalian cells) were in agreement showing mutagenic activity of 2,4-D in the concentration range higher than usually used in establishing plant tissue culture (greater than 5 micrograms/ml).

Gonzalez M, Soloneski S, Reigosa MA, Larramendy ML. 2005 Mar. Genotoxicity of the herbicide 2,4-dichlorophenoxyacetic & a commercial formulation, 2,4-dichlorophenoxyacetic acid dimethylamine salt. I. Evaluation of DNA damage & cytogenetic endpoints in Chinese Hamster ovary (CHO) cells.Toxicol In Vitro 19:289-97.
Abstract: Genotoxicity of the 2,4-dichlorophenoxyacetic acid (2,4-D) and a commercially-used derivative, 2,4-D dimethylamine salt (2,4-D DMA), was evaluated in CHO cells using SCE and single cell gel electrophoresis (SCGE) assays. Log-phase cells were treated with 2.0-10.0mug/ml of herbicides and harvested 24 and 36h later for SCE analysis. Both agents induced significant dose-dependent increases in SCE, regardless of the harvesting time (2,4-D: r=0.98 and r=0.88, P<0.01, for 24 and 36h harvesting times; 2,4-D DMA: r=0.97 and r=0.88, P<0.01, for 24 and 36h harvesting times). Neither test compound altered cell-cycle progression or proliferative replication index (P>0.05), but the higher doses of both compounds reduced the mitotic index of cultures harvested at 24 and 36h (P<0.05). A 90-min treatment with 2.0-10.0mug/ml 2,4-D and 2,4-D DMA produced dose-dependent increases in the frequency of DNA-strand breaks detected in the SCGE assay, both in cultures harvested immediately after treatment and in cultures harvested 36h later. The doses of 2,4-D and 2,4-D DMA were equally genotoxic in all of the assays. The results indicate that 2,4-D induces SCE and DNA damage in mammalian cells, and should be considered as potentially hazardous to humans.

Lueken A, Juhl-Strauss U, Krieger G, Witte I . 2004 Feb 28. Synergistic DNA damage by oxidative stress (induced by H2O2) and nongenotoxic environmental chemicals in human fibroblasts. Toxicol Lett 147:35-43.
Abstract: Genotoxic combination effects of oxidative stress (induced by H2O2) and eight nongenotoxic environmental chemicals (4-chloroaniline, 2,3,4,6-tetrachlorophenol, lindane, 2,4-dichloroacetic acid (2,4-D), m-xylene, glyphosate, nitrilotriacetic acid and n-hexanol) were determined in human fibroblasts. Genotoxicity was measured quantitatively by the single cell gel electrophoresis assay. The nongenotoxic chemicals were used in non cytotoxic concentrations. H2O2 was used in concentrations producing low (50 microM) and no cytotoxicity (40 microM). All environmental chemicals acted in a synergistic way with H2O2 except DMSO which effectively inhibited H2O(2)-induced DNA damage. The most effective enhancers were 4-chloroaniline, 2,3,4,6-tetrachlorophenol, m-xylene, and n-hexanol. Synergistic effects of hexanol/H2O2 were still evident at a

concentration of 0.09 noec (no observed effect concentration). In contrast to synergistic DNA damage in the cell antagonism was found measuring DNA breakage in isolated PM2 DNA. From the results we concluded that synergisms between H2O2 and nongenotoxic chemicals may be a general phenomenon which is not observed on the level of isolated DNA. [***So <u>even allegedely non-genotoxic chemicals are genotoxic</u>**, under oxidative damage stress--a ubiquitous condition. That would show EPA and the industry's claim of 2,4-D's non-mutagenicity to be false ...if it weren't already overwhelmingly (~25 to 1) shown to be false!]*

Micic M, Bihari N, Mlinaric-Rascan I. 2004. Influence of herbicide, 2,4-dichlorophenoxy acetic acid, on haemocyte DNA of in vivo treated mussel. Journal of Experimental Marine Biology and Ecology 311:157-169.
Abstract: The influence of the herbicide 2,4-dichlorophenoxy acetic acid (2,4-D) on haemocyte DNA of in vivo treated mussels Mytilus galloprovincialis has been investigated by flow cytometry and epifluorescence microscopy. Haemocyte proliferation and atypical flow cytometric DNA histograms were observed in mussels treated with 20 and 100 mug/g of 2,4-D. The stimulation of proliferation by 2,4-D was also obvious by DNA labelling with BrdU followed by FITC conjugated anti-BrdU MoAb visualised by epifluorescence microscopy. An apoptotic sub-G, peak resulted in mussels that were exposed to higher doses of herbicide at 100 and 500 mug/g as well as subpopulation could be detected by flow cytometric analysis. In these experiments morphological changes characteristic for apoptotic cells were looked for by fluorescence microscopy. A low percentage of cells in S as well as in G(2)M phase indicating G1 arrest were detected in haemocytes from these mussels that had survived 4 days of 20 mug/g 2,4-D exposure. In addition, sister-chromatid exchanges (SCE) could be seen with the immumolabelling BrdU method. Thus, in vivo treatment and the subsequent uptake of 2,4-D causes serious genetic consequences and raises concerns regarding the potential overall fitness and health effects in mussel populations. (C) 2004 Elsevier B.V. All rights reserved.

Nedopytanska N, Kornuta N, Bagliy E. 2004. Promoting and genotoxic effect of 2,4-D in rats. Toxicol Appl Pharmacol 197:202. [Strangely, even though PubMed indexes this journal, it does not contain this study. Judging by the title, we assume it has positive findings of mutagenicty.]

RESULT NOT STATED/MUTAGENIC

Burroughs BL, Johnson CS, Garry VF. 1996. In vitro micronucleus response of commercial chlorophenoxy herbicides and adjuvants:1-5.
Abstract: (Rough draft without graphs) Chlorophenoxy herbicides, particularly 2,4-D have been epidemiologically associated with excess Non Hodgkins Lymphoma in some studies while not in others (1,2,3,4). In vivo and in vitro studies in animals or in cultured cells of chemically pure chlorophenoxy herbicide do not suggest that these herbicides are notably genotoxic (1 ibid., 5,6,7,8). On the other hand, adjuvants sometimes used in conjunction with these herbicides as spreading and sticking agents have not to our knowledge been examined for genotoxic potential. To test the hypothesis that contaminants in these herbicides or adjuvants might have genotoxic potential, commercial grade chlorophenoxy herbicides, other herbicides and adjuvants were studied. Chemicals used in these in vitro studies were obtained from forest pesticides applicators who use these products in their work. This report is part of a larger laboratory based human population study of forest pesticide applicators.
Keywords:

Tuschl H, Schwab CE. 2004 Aug. Flow cytometric methods used as screening tests for basal toxicity of chemicals. Toxicology in Vitro 18:483-491.
Abstract: Aim of the present study was to evaluate the suitability of flow cytometry to test in vitro effects of toxicants. Flow cytometry offers the possibility to study several parameters simultaneously, e.g. cell cycle modulation, apoptosis and necrosis within the same cell culture. The effects of six compounds (acetaminophen = AAP, isoniazid = INH, digoxin, malathion, paraquat and 2,4-dichlorophenoxy acetic acid = 2,4-D) on cell cycle were investigated in HepG2 cells and the induction of apoptosis/necrosis was analyzed by a spectrum of flow cytometric assays in HepG2, AAH-1 and YAC-1 cells. Early indicators of apoptosis-loss of mitochondrial membrane polarization-as well as later events of the apoptotic process-annexin V binding and DNA fragmentation-were studied. The phases of the cell cycle and the occurrence of a sub-Go peak of apoptotic cells were determined with propidium iodide staining. The present investigation demonstrated good correlations between results obtained by flow cytometric analyses and the IC50 data of the MEIC (= Multicenter Evaluation of In Vitro Cytotoxicity) study. Regarding the short time required for the tests, the possibility of investigating several parameters of cytotoxicity simultaneously and the ease of performance, flow cytometric analyses are well suited for the pre-screening for toxic effects of chemicals. (C) 2004 Elsevier Ltd. All rights reserved. [References: 22] Number of References 22Keywords:

Linnainmaa K. 1984. The effects of hypolipidemic peroxisome proliferators on the induction of sister chromatid exchanges. Basic Life Sci 29 Pt B:965-74.

AMBIGOUS RESULT/MUTAGENIC

Linnainmaa K. 1984 Jun. Induction of sister chromatid exchanges by the peroxisome proliferators 2,4-D, MCPA, and clofibrate in vivo and in vitro. Carcinogenesis 5:703-7.
Abstract: Phenoxy acid herbicides 2,4-dichlorophenoxyacetic acid (2,4-D) and 4-chloro-2-methylphenoxyacetic acid (MCPA) have been found to induce proliferation of peroxisomes in the liver cells of rodents in the same manner as the hypolipidemic drug clofibrate. Both phenoxy acid herbicides and clofibrate (ethyl-alpha-p- chlorophenoxyisobutyrate) are suspected carcinogens. The present study reports the effect of these agents on the induction of sister chromatid exchange (SCE) in the blood lymphocytes of exposed rats (100 mg/kg with 2,4-D and MCPA, 200 mg/kg with clofibrate for 2 weeks in one intragastric dose/day), in the bone marrow cells of exposed Chinese hamsters (100 mg/kg, treatments as above), and in Chinese hamster ovary (CHO) cells in vitro (10(-5), 10(-4), and 10(-3) M, for 1 h). In the experiments in vitro, the effects of purified 2,4-D and MCPA phenoxy acids were studied, in addition to those of the commercial herbicide formulations and clofibrate. No increase of SCE frequency was observed in the blood lymphocytes of the exposed rats in comparison with the controls. In the bone marrow cells of the exposed Chinese hamsters, a slight increase of SCE was found in the group treated with MCPA but not in the groups treated with 2,4-D or clofibrate. A slight increase in the number of SCEs was characteristic of all the treated CHO cell cultures, both with and without a rat liver microsomal activation system (S9 mix). No clear dose-related effects, however, could be discerned with any of the compounds, and no differences in the SCE induction were observed between the commercial herbicide products and the purified phenoxy acetic acids. The present results support the data which indicate that 2,4-D, MCPA, and clofibrate do not act as direct DNA-damaging agents.
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NEGATIVE RESULTS/MUTAGENIC

Charles JM, Cunny HC, Wilson RD, Bus JS, Lawlor TE, Cifone MA, Fellows M, Gollapudi B. 1999 Jul 21. Ames assays and unscheduled dna synthesis assays on 2,4-dichlorophenoxyacetic acid and its derivatives. Mutation Research-Genetic Toxicology & Environmental Mutagenesis 444: 207-216.
Abstract: 2,4-Dichlorophenoxyacetic acid and several of its derivatives (collectively known as 2,4-D) are herbicides used to control a wide variety of broadleaf and woody plants. The genetic toxicity in vitro of 2,4-D and seven of its salts and esters were examined by employing gene mutation in bacteria (Ames test) and induction of DNA damage and repair in rat hepatocytes. In addition, an in vivo unscheduled DNA synthesis (UDS) assay was performed on 2,4-D. There were no indications of genotoxic potential for 2,4-D acid, or any of its derivatives, in these assays. These results are consistent with the reported lack of carcinogenic potential for 2,4-D in both mice and rats. (C) 1999 Elsevier Science B.V. All rights reserved. [References: 21] Number of References 21
Keywords:
Gollapudi BB, Charles JM, Linscombe VA, Day SJ, Bus JS. 1999 Jul 21. Evaluation of the genotoxicity of 2,4-dichlorophenoxyacetic acid and its derivatives in mammalian cell cultures. Mutation Research-Genetic Toxicology & Environmental Mutagenesis 444:217-225.
Abstract: 2,4-Dichlorophenoxyacetic acid and its derivatives (collectively known as 2,4-D) are herbicides used to control a wide variety of broadleaf and woody plants. The genetic toxicity of an ester (2,4-D 2-butoxyethylester) and two salts (2,4-D isopropylamine and 2,4-D triisopropanolamine) was investigated in cultured mammalian cells. The end points used were the induction of chromosomal aberrations in primary cultures of rat lymphocytes and forward mutations at the HGPRT locus of Chinese hamster ovary cells. There was no evidence of genotoxicity for the test materials in the experimental systems used. These results were consistent with the general lack of genotoxic potential for 2,4-D in a number of other test systems. (C) 1999 Elsevier Science B.V. All rights reserved. [References: 11] Number of References 11
Keywords:
Charles JM, Cunny HC, Wilson RD, Ivett JL, Murli H, Bus JS, Gollapudi B. 1999 Jul 21. In vivo micronucleus assays on 2,4-dichlorophenoxyacetic acid and its derivatives. Mutation Research-Genetic Toxicology & Environmental Mutagenesis 444:227-234.
Abstract: The potential for 2,4-D and seven of its salts and esters to induce cytogenetic abnormalities in mammalian cells in vivo was investigated in the mouse bone marrow micronucleus test. All the test materials were administered to male and female mice by oral gavage and the frequencies of micronucleated polychromatic erythrocytes (MN-PCE) in the bone marrow were determined at intervals of 24, 48 and 72 h following dosing. There were no significant increases in the incidence of MN-PCE in the treated mice at any of the bone marrow sampling times. These results are consistent with the reported lack of in vitro genetic toxicity for these materials in various in vitro genotoxicity assays as well as the absence of carcinogenic potential for 2,4-D in both mice and rats. (C) 1999 Elsevier Science B.V. All rights reserved. [References: 23] Number of References 23 Keywords:

Linnainmaa K. 1983. Sister chromatid exchanges among workers occupationally exposed to phenoxy acid herbicides 2,4-D and MCPA. Teratog Carcinog Mutagen 3:269-79.
Abstract: The induction of sister chromatid exchanges (SCEs) was studied in the peripheral lymphocytes of workers spraying foliage in forestry with phenoxy acid herbicides 2,4-dichlorophenoxyacetic acid (2,4-D) and 2-methyl-4-chlorophenoxyacetic acid (MCPA) or their mixtures. In order to follow possible exposure-related changes in the frequencies of SCEs, three successive blood samples were taken from 50 male sprayers during the spraying season of July-October, 1981. In addition, 15 control subjects not working with herbicides were included in the study. The actual exposure levels of the exposed subjects were estimated by measuring the concentrations of 2,4-D and MCPA in the urine of the sprayers. Enough cells for the SCE analysis were obtained from 35 herbicide workers and 15 control subjects. The concentrations of 2,4-D and MCPA in the urine samples after exposure varied from 0.00 to 10.99 mg/l. No significant differences in the frequencies of SCEs were observed in samples taken before, during, or after the exposure. Furthermore, the means of SCEs in a nonexposed control group of 15 subjects fell in the same range as those of the exposed subjects. A difference in the means of SCEs was observed between nonsmokers and smokers, smokers having significantly higher mean values than nonsmokers. The results of the present study add support to the earlier data indicating that 2,4-D and MCPA do not act as direct DNA-damaging agents.

Knapp GW, Setzer RW, Fuscoe JC. 2003. Quantitation of aberrant interlocus t-cell receptor rearrangements in mouse thymocytes and the effect of the herbicide 2,4-dichlorophenoxyacetic acid. Environmental & Molecular Mutagenesis 42:37-43.
Abstract: Small studies in human populations have suggested a correlation between the frequency of errors in antigen receptor gene assembly and lymphoid malignancy risk. In particular, agricultural workers exposed to pesticides have both an increased risk for lymphoma and an increased frequency of errors in antigen receptor gene assembly. In order to further investigate the potential of such errors to serve as a mechanistically based biomarker of lymphoid cancer risk, we have developed a sensitive PCR assay for quantifying errors of V(D)J recombination in the thymocytes of mice. This assay measures interlocus rearrangements between two T-cell receptor loci, V-gamma and J-beta, located on chromosomes 13 and 6, respectively. The baseline frequency in four strains of mice was determined at several ages (2-8 weeks of aged and was found to be stable at similar to 1.5 X 10(-5) per thymocyte. Strain AKR, which has a high susceptibility to T-cell lymphomas, did not show an elevated frequency of aberrant V(D)J events. We used this assay to examine the effects of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) on the frequency of these events. Female B63F1 mice, 27 days of age, were exposed to 2,4-D by gavage at doses of 0, 3, 10, 30, and 100 mg/ kg/day for 4 successive days and sacrificed on day 5. Thymus DNA was isolated and examined for illegitimate V(D)J recombination-mediated gene rearrangements. In addition, pregnant mice were exposed to 2,4-D and thymocytes from the offspring examined at 2 weeks of age. No significant increase in aberrant V(D)J rearrangements was found, indicating that under these conditions 2,4-D does not appear to effect this important mechanism of carcinogenesis. [References: 40] Number of References 40 Keywords:
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OTHER CANCER MECHANISMS

Mechanistic are almost always experimental (prospective, controlled variables) studies. In this sub-category too the positive results overwhelmingly outweigh the negative results. It is thus notable that all published studies of 2,4-D's mechanism of carcinogenicity overwhelmingly and robustly find that 2,4-D plays several pro-carcinogenic roles. It is notable, as we stated above, that the immune system is a target 2,4-D, because both the epidemiologic and the mechanistic literature strongly support 2,4-D causing immune cancers. These results stress the evaluation of the experimental animal studies that you rely on in classifying 2,4-D as having insufficient evidence of carcinogenicity; and the main question that naturally arises is what is the quality of your evidence. In short, how many of the studies that you are relying on are published in independent journals? Please respond directly.

POSITIVE RESULTS/OTHER CANCER MECHANISMS

Blakley BR, Gagnon JM, Rousseaux CG. 1992 Aug. The effect of a commercial 2,4-D formulation on chemical- and viral-induced tumor production in mice. J Appl Toxicol 12:245-9.
Abstract: Male CD-1 mice were exposed to a commercial formulation of 2,4-dichlorophenoxyacetic acid (2,4-D), the amine derivative, in the drinking water at concentrations ranging from 0 to 0.163% of the formulated product, equivalent to approximately 0-50 mg kg-1 day-1 2,4-D content. The effect of 2,4-D on urethan-induced pulmonary adenoma formation was evaluated following a 105-day exposure. Urethan-induced sleeping times observed following an i.p. injection of urethan (1.5 mg g-1) after 3 weeks of 2,4-D exposure were not altered by 2,4-D, indicating that 2,4-D did not influence

urethan elimination. Pulmonary adenoma production, which was evaluated 84 days after urethan injection, was enhanced by 2,4-D exposure but had no effect on tumor size. The effect of 2,4-D on the incidence of spontaneous murine lymphocytic leukemia was evaluated during the 365-day treatment period. Mortality associated with the leukemia virus was not altered by 2,4-D treatment. Exposure to this commercial 2,4-D product at moderately high levels of exposure may modify the development or expression of certain tumors in CD-1 mice. The mechanism of the co-carcinogenic or tumor-promoting activity associated with 2,4-D exposure remains to be determined.

Figgs LW, Holland NT, Rothmann N, Zahm SH, Tarone RE, Hill R, Vogt RF, Smith MT, Boysen CD, Holmes FF, VanDyck K, Blair A. 2000 Apr. Increased lymphocyte replicative index following 2,4-dichlorophenoxyacetic acid herbicide exposure. Cancer Causes Control 11:373-80.
Abstract: OBJECTIVE: Evaluate peripheral blood lymphocyte proliferation (replicative index:RI) and micronuclei frequency (MF) among 2,4-D herbicide applicators. METHODS: Twelve applicators spraying only 2,4-D provided a blood and urine specimen upon enrollment, several urine samples during the spraying season, and a blood specimen at the study's end. Nine controls provided blood and urine specimens upon enrollment and at the study's end. Gas chromatography/tandem mass spectroscopy determined urinary 2,4-D levels and standard in-vitro assays determined RI and MF scores. Applicator RI and MF were compared before and after spraying and with controls. RESULTS: Applicators contributed 45 urine specimens with concentrations ranging from 1.0 to 1700 (microg 2,4-D/g creatinine/L urine) that logarithmically (ln) increased as spraying time increased. Applicator RI increased after spraying (p = 0.016), independent of tobacco and alcohol use, and demonstrated a weak dose-response with increasing urinary 2,4-D levels (p = 0.15). Among 2,4-D applicators, pre-exposure complete blood counts and lymphocyte immunophenotypes were not significantly different from post-exposure measurements. CONCLUSION: Urinary 2,4-D concentration, an exposure biomarker, may be associated with lymphocyte replicative index, a cell proliferation biomarker.
[ALSO IN 'POSITIVE' RESULTS/MUTAGENIC']

Holland NT, Duramad P, Rothman N, Figgs LW, Blair A, Hubbard A, Smith MT. 2002 Nov 26. Micronucleus frequency and proliferation in human lymphocytes after exposure to herbicide 2,4-dichlorophenoxyacetic acid in vitro and in vivo. Mutation Research-Genetic Toxicology & Environmental Mutagenesis 521:165-178.
Abstract: Widespread use of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) and its association with non-Hodgkin's lymphoma (NHL) and other cancers has raised public concern. Here, micronucleus (MN) formation has been used as a biomarker of genotoxicity, and replicative and mitotic indices (MIs) as biomarkers of cell cycle kinetics in human lymphocytes. Cells were cultured either as whole blood or isolated lymphocytes and treated with pure or commercial forms of 2,4-D at doses between 0.001 and 1 mM for 48 h. Exposure to 2,4-D produced a minimal increase in MN in whole blood and even smaller one in isolated lymphocyte cultures. This induction took place only at levels approaching cytotoxicity and was accompanied by a significant inhibition of replicative index (RI). At a low (0.005 mM) dose of commercial 2,4-D, a small, marginally significant increase in RI (12-15%) was found in two independent sets of experiments (P = 0.052). Additionally, we found that lymphocyte RI was more affected by commercial 2,4-D containing 9.4% of the chemically pure 2,4-D, than with an equal concentration of the latter suggesting that other ingredients present in the commercial pesticide may be responsible or may enhance the effect of 2,4-D. Mitotic index, however, did not show any significant change with either commercial or pure 2,4-D. The lymphocytes of 12 male applicators exposed solely to 2,4-D during a 3-month period had a significantly higher RI than the same group prior to exposure and than a control group (P < 0.01), in accordance with the in vitro finding of increased RI at low doses. (C) 2002 Elsevier Science B.V. All rights reserved. [References: 61] Number of References 61Keywords:
[ALSO IN 'MUTAGENIC/POSITIVE' RESULTS. **NOTE THE LOW (5 NanoMOLE) DOSE EFFECT**]

Kaioumova D, Susal C, Opelz G. 2001 Jan. Induction of apoptosis in human lymphocytes by the herbicide 2,4-dichlorophenoxyacetic acid. Hum Immunol 62:64-74.
Abstract: Dimethylammonium salt of 2,4-dichlorophenoxyacetic acid (DMA-2,4-D) is a widely used herbicide that is considered moderately toxic. In the present study we found that DMA-2,4-D is able to cause apoptosis in peripheral blood lymphocytes of healthy individuals and Jurkat T cells. Apoptosis induced by DMA-2,4-D was dose and time dependent, independent of Fas, TNF receptor 1 or the aromatic hydrocarbon receptor, and involved disruption of the mitochondrial transmembrane potential and activation of caspase-9. ZVAD-FMK, a broad-spectrum inhibitor of caspases, blocked DMA-2,4-D-induced apoptosis completely. While an inhibitor of caspase-9, as well as caspase-9 and caspase-3 inhibitors in combination, strongly blocked DMA-2,4-D-induced apoptosis, an inhibitor of caspase-3 had a moderate inhibitory effect. Unlike Fas-mediated apoptosis, the initiator caspase, caspase-8, was not involved in DMA-2,4-D-induced apoptosis. Transfection of Jurkat cells with Bcl-2 prevented DMA-2,4-D-induced disruption of the mitochondrial transmembrane potential and led to a complete blockage of apoptosis. Our data indicate that DMA-2,4-D kills human lymphocytes by initiating apoptosis via a direct effect on mitochondria. The activation of caspases occurs downstream of mitochondrial damage, and the dysfunction of mitochondria appears to be sufficient for triggering all downstream events leading to apoptosis.

Lin N, Garry VF. 2000. *In vitro* studies of cellular and molecular developmental toxicity of adjuvants, herbicides, and fungicides commonly used in Red River Valley, Minnesota. Journal of Toxicology & Environmental Health - Part A 60:423-

439.
Abstract: Recent epidemiologic studies showed increased frequency of birth defects in pesticide applicators and general population of the Red River Valley, Minnesota. These studies further indicated that this crop growing area used more chlorophenoxy herbicides and fungicides than elsewhere in Minnesota. Based on frequency of use and known biology, certain herbicides, pesticide additives, fungicides, and mycotoxins and suspect agents. To define whether these agents affect developmental endpoints *in vitro*, 16 selected agrochemicals were examined using the MCF-7 breast cancer cell line. In the flow cytometric assay, cell proliferation in this estrogen-responsive cell line indicates xenobiotic-mediated estrogenic effects. Cell viability, morphology, ploidy, and apoptosis were incorporated in this assay. Data showed that the adjuvants X-77 and Activate Plus induced significant cell proliferation at 0.1 and 1 ìg/ml. The commerical-grade herbicide 2,4-D LV4 and 2,4-D amine induced cell proliferaton at 1 and 10 ìg/ml. The reagent-grade 2,4-D products failed to induce proliferation over the same concentration range, suggesting that other ingredients in the commercial products, presumably adjuvants, could be a factor in these results. The fungicides triphenyltin and mancozeb induced apoptosis at concentration of 4.1 ìg/ml (10-5 M). Data provide a mechanistic step to understanding human reproductive and developmental effects in populations exposed to these agrochemicals, and initiative to focusing limited resources for future use *in vivo* animal developmental toxicity studies.
Keywords:

Merillon JM, Filali M, Duperon P, Montagu M, Chenieux JC, Rideau M. 1995 Jul-1995 Aug 31. Effect of 2,4-dichlorophenoxyacetic acid and habituation on lipid and protein composition of microsomal membranes from periwinkle cell suspensions. Plant Physiology & Biochemistry 33:443-451.
Abstract: Investigations on biochemical changes associated with habituation in plant cells and tissues grown in vitro are still Limited. In the present work, we have compared the lipid and protein composition of microsomal membranes prepared from two Catharanthus roseus cell lines: a 2,4-dichlorophenoxyacetic acid (2,4-D)-dependent line and a fully-habituated line selected from the former. In order to distinguish changes associated with habituation from those associated with 2,4-D treatment, each line was grown for one passage in medium with or without 2,4-D. The auxin Provoked a lower amount of phospholipid, a higher free-sterol to phospholipid ratio, and a decreased fluidity in microsomal membranes, all parameters usually associated with cell senescence. On the other hand, habituation decreased the free sterol to phospholipid ratio, increased the oleic add to linoleic acid ratio and the sitosterol to campesterol ratio. The fluidity of the membranes from habituated cells increased. Habituation, as well as treatment of the cells with 2,4-D, changed the polypeptide profiles of the microsomal membranes. The data lead to the conclusion that membranes are targets for biochemical changes associated with habituation. They also support the hypothesis that some similarities exist between habituated cells and animal tumour cells. [References: 38] Number of References 38
Keywords:

Palmeira CM, Moreno AJ, Madeira VMC. 1994 Jul. Interactions of herbicides 2,4-d and dinoseb with liver mitochondrial bioenergetics. Toxicology & Applied Pharmacology 127:50-57.
Abstract: The herbicides 2,4-D (2,4-dichlorophenoxyacetic acid) and dinoseb (2-sec-butyl-4,6-dinitrophenol), were tested in mitochondria because they are putative toxins to the organisms. To understand the toxic mechanisms involved, we have determined if mitochondrial bioenergetic functions are affected. Dinoseb partially inhibits uncoupled respiration, reflecting its limited interaction with the mitochondrial redox chain at the level of succinate dehydrogenase and cytochrome c reductase (complex III). Additionally, it increased the rate of state 4 oxygen consumption, stimulated ATPase activity, induced permeabilization of membrane mitochondria to H+, and depressed Delta Psi. These data characterize dinoseb as a classical proton uncoupler. The herbicide 2,4-D decreased Delta Psi as a function of concentration and the rate of repolarization was also progressively decreased. State 3 and uncoupled respiration were depressed by approximately the same extent (60%), ruling out interactions on phosphorylation assembly independent of the redox chain. The herbicide strongly inhibited succinate dehydrogenase and cytochrome c reductase (complex III), whereas cytochrome c oxidase was not affected. Additionally, 2,4-D also uncoupled mitochondria at concentrations 1000-fold higher than those required for a similar dinoseb effect. This study therefore suggests that dinoseb- and 2,4-D-induced cellular damage, as we have reported before, is putatively preceded by injury upon bioenergetic functions of mitochondria. (C) 1994 Academic Press, Inc. [References: 42] Number of References 42 Keywords:

Faustini A, Settimi L, Pacifici R, Fano V, Zuccaro P, Forastiere F. 1996. Immunological changes among farmers exposed to phenoxy herbicides - Preliminary observations. Occupational & Environmental Medicine 53:583-585.
Abstract: Objectives-To evaluate short term immunological changes after agricultural exposure to commercial formulations of chlorophenoxy herbicides. Methods-Blood samples were collected hom 10 farmers within seven days before exposure, one to 12 days after exposure, and again 50 to 70 days after exposure. Whole blood was used to count lymphocyte subsets with monoclonal antibodies. Peripheral blood mononuclear (PBM) cells were used to measure natural killer (NK) cell activity and lymphocyte response to mitogenic stimulations. Values before exposure were used as reference. Results-in comparison with concentrations before exposure, a significant reduction was found one to 12 days after exposure in the following variables ($P < 0.05$): circulating helper (CD4) and suppressor T cells (CD8), CD8 dim, cytotoxic T lymphocytes (CTL), natural killer cells (NK), and CD8 cells expressing the surface antigens HLA-DR (CD8-DR), and lymphoproliferative response to mitogen stimulations. All immunological values found 50-70 days after exposure

were comparable with concentrations before exposure, but mitogenic proliferative responses of lymphocytes were still significantly decreased. Conclusions-According to our data agricultural exposure to commercial 2,4-dichlorophenoxyacetic acid (2,4-D) and 4-chloro-2-methylphenoxyacetic acid (MCPA) formulations may exert short term immunosuppressive effects. Further studies should clarify whether the immunological changes found may have health implications and can specifically contribute to cancer aetiology.Keywords:
[IMMUNE PARAMETERS INCREASED AFTER EXPOSURE, THEN DECREASED--A HINT OF CAUSATION:]

Jenssen D, Renberg L. 1976 Aug. Distribution and cytogenetic test of 2,4-D and 2,4,5-T phenoxyacetic acids in mouse blood tissues. Chem Biol Interact 14:279-89.
Abstract: The phenoxyacetic acids 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), extensively used as herbicides, were tested for cytogenetic effects by means of induced micronuclei in erythrocytes of mouse bone marrow. Because of the hig experimental resolution power this is a particularly suitable test system for the detection of weak chromosome breaking activity in mammals. The cytogenetic tests were supplemented with chemical analyses of the concentration the the test substances reaching the target cells...

Schop RN, Hardy MH, Goldberg MT. 1990 Nov. Comparison of the activity of topically applied pesticides and the herbicide 2,4-D in two short-term in vivo assays of genotoxicity in the mouse. Fundam Appl Toxicol 15:666-75.
Abstract: Genotoxicity of eight topically applied compounds was determined using the bone marrow micronucleus (MN) test and hair follicle nuclear aberration (NA) assay in CD1 mice. Twenty-four hours after a single treatment, cyclophosphamide (CY), applied at doses corresponding to 1/4, 1/8, 1/16, and 1/32 of the published dermal LD50, and N-methyl-N-nitrosourea (MNU), applied at 1/4, 1/8, and 1/16 of the published dermal LD50, were found to increase the incidence of NA in a dose-dependent manner. The frequency of MN was significantly increased only at the highest dose of CY. Using the same protocol, six pesticides applied in dimethyl sulfoxide (DMSO) at doses of 1/8, 1/16, and 1/32 of the dermal LD50 were investigated. Aminocarb and chlordane induced a dose-dependent increase in the frequency of NA, while there was an observed increase in NA incidence at only the highest doses of dichlorvos (DDVP), 4,4'-DDT (DDT), and 2,4-dichlorophenoxyacetic acid (2,4-D). No effect was observed with fenitrothion on nuclear aberrations in hair follicles. Except for the highest dose of chlordane, none of the pesticides tested positive in the bone marrow micronucleus test. Serum cholinesterase levels were reduced to 70 +/- 4.7% of the DMSO control level with DDVP, 57 +/- 8.2% with aminocarb, and 60.3 +/- 4.8% with fenitrothion, indicating some systemic activity with these topically applied agents. The data suggest that aminocarb, chlordane, DDVP, DDT, and 2,4-D are genotoxic as determined by the NA assay and that this assay may be more useful in detecting topically applied genotoxic agents than the more often used bone marrow micronucleus test.

Tuschl H, Schwab C. 2003 Mar. Cytotoxic effects of the herbicide 2,4-dichlorophenoxyacetic acid in hepg2 cells. Food & Chemical Toxicology 41:385-393.
Abstract: 2,4-Dichlorophenoxyacetic acid (2,4-D) and its derivatives are herbicides widely used to control the growth of broadleaf and woody plants. Although 2,4-D is well known to be moderately toxic, little information is available on the mechanisms of its toxicity. Results on carcinogenicity, genotoxicity and mutagenicity are contradictory, but neurotoxic, immunosuppressive and hepatotoxic effects have been defined. The aim of the present study was to investigate the cytotoxic effects of 2,4-D on a human hepatoma cell line. HepG2 cells were treated with different concentrations of 2,4-D, and cell viability, induction of apoptosis/necrosis and cell cycle phases were determined. Apoptosis was detected in flow cytometric light scatter histograms, the annexin V assay, the determination of DNA strand breaks with the TUNEL assay and the occurrence of a sub G(0) peak after propidium iodide (PI) staining. The induction of apoptosis by 2,4-D was accompanied by a disruption of the mitochondrial membrane potential as verified by staining with the cationic JG-1 probe. In addition, 2,4-D affected the cell cycle in a concentration-dependent manner. Our investigation suggested that 2,4-D exerts its cytotoxic effects by the induction of apoptosis via a direct effect on the mitochondrial membrane potential. (C) 2002 Elsevier Science Ltd. All rights reserved. [References: 26] Number of References 26 Keywords:

NEGATIVE RESULT/CANCER MECHANISMS/OTHER

Charles JM, Bond DM, Jeffries TK, Yano BL, Stott WT, Johnson KA, Cunny HC, Wilson RD, Bus JS. 1996 Oct. Chronic dietary toxicity/oncogenicity studies on 2,4-dichlorophenoxyacetic acid in rodents. Fundam Appl Toxicol 33:166-72.
Abstract: Forms of 2,4-dichlorophenoxyacetic acid (collectively known as 2,4-D) are herbicides used to control a wide variety of broadleaf and woody plants. Doses in the 2-year chronic/oncogenicity rat study were 0, 5, 75, and 150 mg/kg/day. The chronic toxicity paralleled subchronic findings, and a NOEL of 5 mg/kg/day was established. A slight increase in astrocytomas observed (in males only) at 45 mg/kg/day in a previously conducted chronic rat study was not confirmed in the present study at the high dose of 150 mg/kg/ day. Doses in the 2-year mouse oncogenicity studies were 0, 5, 150, and 300 mg/kg/day for females and 0, 5, 62.5, and 125 mg/ kg/day for males. No oncogenic effect was noted in the study. In summary, the findings of these studies indicate low chronic toxicity of 2,4-D and the lack of oncogenic response to 2,4-D following chronic dietary exposure of 2,4-D in the rat & mouse.
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CANCER/MECHANISM/PEROXISOME PROLIFERATION

2,4-D is an undisputed peroxisome proliferater (PP), but PP's role in cancer in different species is the subject of a legitimate and vigorous scientific debate. Yet it is *not disputed* that PPs, and thus 2,4-D, are risk factors for cancer in various vertebrate and other animal species, if not in humans.

POSITVE RESULTS/CANCER MECHANISM/PEROX. PROLIF.

Ge R, Tao L, Kramer PM, Cunningham ML, Pereira MA. 2002. Effect of peroxisome proliferators on the methylation and protein level of the c-myc protooncogene in B6C3F1 mice liver. J Biochem Mol Toxicol 16:41-7.
Abstract: Peroxisome proliferators in general are nongenotoxic mouse liver carcinogens for which DNA hypomethylation and altered gene expression are proposed mechanisms. Therefore, the peroxisome proliferators 2,4-dichlorophenoxyacetic acid (2,4-D), dibutyl phthalate (DBP), gemfibrozil, and Wy-14,643 were evaluated for the ability to alter the methylation and expression of the c-myc protooncogene. Male B6C3F1 mice were administered for 6 days in their diet Wy-14,643 (5-500 ppm), 2,4-D (1,680 ppm), DBP (20,000 ppm), or gemfibrozil (8,000 ppm). All four peroxisome proliferators caused hypomethylation of the c-myc gene in the liver. Wy-14,643 appeared to be the most efficacious with a threshold between 10 and 50 ppm. The level of the c-myc protein was increased by Wy-14,643, but not the other peroxisome proliferators. When female B6C3F1 mice received a two-thirds partially hepatectomy and 16 h later were administered 50 mg/kg Wy-14,643 by gavage, hypomethylation of the gene occurred 24 h later. Hypomethylation was not found in mice that received Wy-14,643 following a sham operation. Hypomethylation of the c-myc gene within 24 h of administering Wy-14,643 after a partial hepatectomy but not after a sham operation supports the hypothesis that the peroxisome proliferators prevent methylation of hemimethylated sites formed by DNA replication.

Vainio H, Nickels J, Linnainmaa K. 1982 Mar. Phenoxy acid herbicides cause peroxisome proliferation in Chinese hamsters. Scand J Work Environ Health 8:70-3. Abstract: An increase in either the size or amount of peroxisomes was obtained in the liver cells of Chinese hamsters after the animals were exposed to the phenoxy herbicides 2,4-dichlorophenoxyacetic acid (2,4-D) or 4-chloro-2-methylphenoxyacetic acid (MCPA). At the dose level studied, 2,4-D was found to be more potent than MCPA in increasing the number of peroxisomes. A phenoxy acid derivative, clofibrate, one of the peroxisome proliferators known to possess carcinogenic properties in rodents, appeared to be still more potent in inducing peroxisome proliferation than either of the herbicides studied. Further investigations are warranted to clarify the significance of peroxisome proliferation to the toxicity of phenoxy herbicides.

Biochem Pharmacol. 1983 Sep 15;32(18):2775-9. Hypolipidemia and peroxisome proliferation induced by phenoxyacetic acid herbicides in rats. Vainio H, Linnainmaa K, Kahonen M, Nickels J, Hietanen E, Marniemi J, Peltonen P.
Male Wistar rats were treated daily by gavage with two phenoxy herbicides, 2,4-dichlorophenoxyacetic acid (2,4-D)(100-200 mg/kg body wt) and 4-chloro-2-methylphenoxyacetic acid (MCPA) (100-200 mg/kg body wt), and with the chemically different glyphosate N-phosphonomethyl glycine (300 mg/kg body wt) 5 days per week for 2 weeks. A hypolipidemic drug, clofibrate [ethyl-2-(4-chlorophenoxy)-2-methylpropionate], which is structurally related to phenoxy acids, was used as a positive control (200 mg/kg body wt). 2,4-D and MCPA had several effects similar to those of clofibrate: all three compounds induced proliferation of hepatic peroxisomes, decreased serum lipid levels, and increased hepatic carnitine acetyltransferase and catalase activities. 2,4-D and MCPA, but not clofibrate, decreased lipoprotein lipase activity in the adipose tissue to about a third of the control value but did not change the lipoprotein lipase activity in the heart muscle. The data suggest that these compounds cause hypolipidemia not by enhancing the storage of peripheral lipids in adipose tissue but by preferentially increasing lipid utilization in the liver. Glyphosate caused no peroxisome proliferation or hypolipidemia, suggesting that these effects are associated with the structural similarity between phenoxy acid herbicides and clofibrate. PMID: 6626247 [PubMed - indexed for MEDLINE]

Acta Pharmacol Toxicol (Copenh). 1983 Aug;53(2):103-12. Effects of phenoxyherbicides and glyphosate on the hepatic and intestinal biotransformation activities in the rat. Hietanen E, Linnainmaa K, Vainio H.
The effects of phenoxyacid herbicides 2,4-D (2,4-dichlorophenoxyacetic acid) and MCPA (4-chloro-2-methylphenoxyacetic acid), clofibrate, and glyphosate on hepatic and intestinal drug metabolizing enzyme activities were studied in rats intragastrically exposed for 2 weeks. The hepatic ethoxycoumarin O-deethylase activity increased about 2-fold with MCPA. Both 2,4-D and MCPA increased the hepatic epoxide hydrolase activity and decreased the hepatic glutathione S-transferase activity. MCPA also increased the intestinal activities of ethoxycoumarin O-deethylase and epoxide hydrolase. Glyphosate decreased the hepatic level of cytochrome P-450 and monooxygenase activities and the intestinal activity of aryl hydrocarbon hydroxylase. Clofibrate decreased the hepatic activities of UDPglucuronosyltransferase with p-nitrophenol or methylumbelliferone as the substrate. Also 2,4-D decreased the hepatic activity of UDPglucuronosyltransferase with p-nitrophenol as the substrate. MCPA decreased the intestinal activities of UDPglucuronosyltransferase with either p-nitrophenol or methylumbelliferone as the substrate. The results

indicate that phenoxyacetic acids, especially MCPA, may have potent effects on the metabolism of xenobiotics. Glyphosate, not chemically related to phenoxyacids, seems to inhibit monooxygenases. Whether these changes are related to the toxicity of these xenobiotics remains to be clarified in further experiments. PMID: 6624478 [PubMed - indexed for MEDLINE]

Ozaki K, Mahler JF, Haseman JK, Moomaw CR, Nicolette ML, Nyska A. 2001 Jul. Unique renal tubule changes induced in rats and mice by the peroxisome proliferators 2,4-dichlorophenoxyacetic acid (2,4-d) and wy-14643. Toxicol Pathol 29:440-450.
Abstract: Peroxisome proliferators are non-mutagenic carcinogens in the liver of rodents, acting both as initiators and promoters. The National Toxicology Program (NTP) conducted a study of several peroxisome proliferators (PPs), including Wyeth (WY)-14643 as a prototypical PP and 2,4-dichlorophenoxyaceti c acid (2,4-D) as a weak PP, in Sprague-Dawley rats, B6C3F1 mice, and Syrian hamsters. In the kidney, an unusual change was observed in the outer stripe of the outer medulla, especially in rats treated with 2,4-D or WY-14643. This change was characterized by foci of tubules that were partially or completely lined by basophilic epithelial cells with decreased cytoplasm and high nuclear density. Changes typical of chronic nephropathy such as interstitial fibrosis or basement membrane thickening were not associated with these foci. Results of immunohistochemical staining for catalase and cytochrome P-450 4A in the kidney indicated increased staining intensity in renal tubular epithelial cells primarily in the region where the affected tubules were observed; however, the altered cells were negative for both immunohistochemical markers. Ultrastructurally, affected cells had long brush borders typical of the P3 tubule segment. The most distinguishing ultrastructural change was a decreased amount of electronlucent cytoplasm that contained few differentiated organelles and, in particular, a prominent reduced volume and number of mitochondria; changes in peroxisomes were not apparent. In addition to the lesion in rats, mice treated with the highest dose of 2, 4-D, but not WY-14643, manifested similar renal tubular changes as seen by light microscopy. Neither chemical induced renal tubular lesions in hamsters. Hepatocellular changes characteristic of PPs were present in all 3 species treated with WY-14643, but not 2,4-D. These results indicate that the rat is the species most sensitive to the nephrotoxic effects of PPs and there is a site specificity to this toxicity related to areas of PP-related enzyme induction. Although 2, 4-D is considered a weak PP for the liver, it was the most effective at inducing renal lesions, indicating that the toxic potency of various PPs will depend on the target organ. [References: 38]
Number of References 38 Keywords:
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NEGATIVE RESULTS/MECHANISM/PEROX. PROLIF.

Abdellatif AG, Preat V, Vamecq J, Nilsson R, Roberfroid M. 1990 Nov. Peroxisome proliferation and modulation of rat liver carcinogenesis by 2,4-dichlorophenoxyacetic acid, 2,4,5-trichlorophenoxyacetic acid, perfluorooctanoic acid and nafenopin. Carcinogenesis 11:1899-902. Abstract: Using an initiation--selection--promotion protocol for induction of liver tumors in Wistar rats, the modulating action of various peroxisome proliferators on neoplasia as well as on selected biochemical parameters was studied. After treatment with diethylnitrosamine (DEN), the animals were subsequently subjected to a selection procedure involving feeding of 2-acetylaminofluorene (2-AAF), and in the middle of the 2-AAF treatment, a single necrogenic dose of carbon tetrachloride. Following a recovery period, the rats were fed a diet containing 0.1% nafenopin (NAF), 0.015% perfluorooctanoic acid (PFOA), 0.05% 2,4-dichlorophenoxyacetic acid (2,4-D), 0.05% 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) or 0.05% phenobarbital (PB) as a positive control. When the animals were killed, 7 months after initiation, the incidence of hepatocellular carcinoma was 83, 33 and 16% in the animals treated with NAF, PFOA or 2,4,5-T respectively. No cancers were observed in controls, or in the 2,4,-D groups. In comparison with controls, NAF and PFOA caused a 60-and 24-fold increase inthe peroxisomal beta-oxidation of fatty acids respectively, but only about a 2-fold increase in the catalase activity, 2,4-D and/or 2,4,5-T were much less active in this respect, giving approximately a doubling in the rate of fatty acid oxidation. The specific activity of D-amino acid and glycolate oxidases were significantly depressed, whereas the urate oxidase levels were apparently unaffected by the NAF and PFOA treatment. The results suggest that the selective induction of peroxisomal fatty acid oxidation is consistent with the hypothesis that imbalance between H2O2 overproduction and its destruction could play a role in the modulation of hepatocarcinogenesis by peroxisome proliferators.

Mikalsen SO, Ruyter B, Sanner T. 1990 Feb 1. Effects of hepatic peroxisome proliferators and 12-O-tetradecanoyl phorbol-13-acetate on catalase and other enzyme activities of embryonic cells in vitro. Biochem Pharmacol 39:527-35. Abstract: The effects of the hepatic peroxisome proliferators (HPPs) clofibrate, di-(2-ethylhexyl)-phthalate (DEHP), mono-(2-ethylhexyl)phthalate (MEHP) and 2,4-dichlorophenoxy acetic acid (2,4-D) on the activities of some peroxisome-associated enzymes and marker enzymes for other organelles, have been studied in primary Syrian hamster embryo (SHE) cells and Wistar rat embryo (WRE) cells. The majority of the cells are fibroblast-like. 12-O-Tetradecanoyl phorbol-13-acetate (TPA) was included as it has been suggested that it may act as a peroxisome proliferator. The specific activities of catalase, fatty acyl-CoA oxidase (FAO) and peroxisomal beta-oxidation were approximately 100-fold lower in the embryonic cells than in rat hepatocytes. Other peroxisome-associated oxidases were not detected. The dihydroxyacetone-phosphate acyltransferase (DHAPAT) activity was comparable to that in rat liver. Marker enzymes for

other organelles had specific activities comparable to rat hepatocytes. Catalase was shown by digitonin titration to be contained in a peroxisome-like compartment in both SHE and WRE cells. Clofibrate, DEHP and MEHP increased the catalase activity, which might suggest peroxisome proliferation. However, the findings that FAO and peroxisomal beta-oxidation did not increase or only very slightly, argue against peroxisome proliferation. 2,4-D and TPA induced no or only a very slight increase in the catalase activity.
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