14 September 2004

USDA Forest Service
ATTN: Rita Morgan, Data Quality Official
Mail Stop 1143
1400 Independence Ave. SW
Washington, DC 20250-1143

Dear Ms. Morgan:

This is a:
Request to the USFS for Correction of Information, under Information Quality Guidelines

(Statement that the Request for Correction of Information is Submitted Under USDA's Information Quality Guidelines)

We submit this Request for Correction of Information under USDA's Information Quality Guidelines; which implement the Dec. 2000 amendments to USC 1501(1) et seq., the Paperwork Reduction Act)--ammendments that are uncodified but known as the Information Quality Act (IQA) or the Data Quality Act (DQA).
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(Requester Contact Information)

Tony Tweedale, for:
The Alliance for the Wild Rockies
P.O. Box 8731
Missoula, MT 59807

tel. 406-721-5420 ttweed@wildrockies.org
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(Description of Information to Correct)

Each of the following three Final Environmental Impact Statements (FEIS) for Forest-wide weed control plans make highly consequential yet incorrect assertions about the chronic health effects of herbicides used for noxious weed control in National Forests. These assertions that are summarized in one table per document (sometimes, the table is the FEIS's sole assessment of the chronic health risks of the herbicides to be used):

- Table 3.5.3, 'Comparison of Harmful Chronic Effects of Herbicides Proposed for Controlling Weeds on the BDNF' (USFS May 2002 'Beaverhead-Deerlodge Ntl. Forest Noxious Weed Control Final EIS & RoD' pg. 3-6);

- Table 4.7, 'Comparison of Herbicide Toxicity' (USFS March 2003 'Bitteroot National Forest Noxious Weed Treatment Project Final EIS', pg. 4-73);

- Table 4.5, 'Comparison of Harmful Chronic Effects of Herbicides Proposed for Controlling Weeds on the S-CNF (USFS Sept. 2003 Salmon-Challis National Forest Noxious Weed Management Program Final EIS', pg. 4-86 to 4-87);
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(Explanation of Noncompliance with OMB and/or USDA Information Quality Guidelines)

The USFS has published many toxicology reviews that significantly underestimate the non-acutely lethal health effects of pesticides. These USFS reviews rely almost entirely (directly, or through a previous USFS risk assessments) on the toxicity tests required to register those pesticides for use under the federal pesticide regulatory statute, FIFRA.

Unfortunately, these registration test requirements (40 CFR 152.1 et seq.), overseen by the U.S. EPA, are performed by companies with conflict of interests. Typically tens to hundreds of millions of dollars are riding on each pesticide being found to be safe for use. Thus such data is not independent of self-interest; i.e. it can not be objective. After a pesticide is marketed, these typically unpublished, un-peer reviewed, self-interested results showing relative safety are gradually refuted; as independent academic researchers in published studies begin to study the real risks. Moreover, there are critical data gaps in these registration tests--i.e. many chronic effects are not required to be tested before registration is granted.

Per se then, these pesticide registration data and data gaps do not meet the guideline designed to meet the objectivity mandate of the DQA (the guideline requires completeness, unbias and accuracy to be deemed objective). Under the DQA and agency Guidelines the Forest Service must ensure the objectivity of the pesticide toxicity data and its dissemination, including the consideration of the claims made in this Request. The National Environmental Policy Act (NEPA) also requires that assessments of environmental impacts be based on adequate and reliable data.

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These three FEIS summary Tables (and other non-acutely lethal herbicide toxicity data the FEIS present) violate the USDA DQA General Guideline for objectivity because they are biased, incomplete and inaccurate. They also violate the 'Supplementary [DQA] Guidelines for the Quality of Scientific Research Information', whose purpose is to establish "..information quality criteria that USDA agencies and offices will follow in developing and reviewing scientific information and disseminating it to the public.". Most of those criteria pertain to the DQA's objectivity requirement. Under these Supplementary Guidelines, all scientific research information before it is released to the public by an agency must be independently peer reviewed, or the agency must assure that the information has already been subjected to the same review (typically by being published in a reputable peer reviewed journal).

The proof that this non-lethal effects data relied on by the USFS is non-objective per the DQA and its Guidelines is that these three FEIS' summary tables rely on pesticide registration data, typically unpublished, whose claims are refuted by hundreds of independently published studies. For each cell of each of these tables we present on average more than four independent (peer-reviewed, published by scientific journals--occasionally by government agencies) studies that support the opposite conclusion of these non-independent studies (the few remaining cells say "Unknown"). See our appendix for these refutations.

Under the DQA Guidelines, results published in an independent, peer-reviewed scientific journal are rebuttaly presumed to be objective data. Our data is almost entirely published in such journals, while the Forest Service's data--which reaches opposite conclusions--is not. Our few studies that are not so published are performed by government agency scientists, whose work and data have undergone similar procedures to assure and quality, such as peer-review and transparency (also, government scientists are independent by nature of their position). Also the DQA Guidelines acknowledge that the 1996 law, USC 300g-1(b)(3)(a), requires that all federal agency actions be based on the best available (sound and objective), peer-reviewed science; and data collected by accepted scientific methods; to the degree that the action relies on science. These three FEIS are not in compliance with these laws and Guidelines.

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Additionally, we believe that information on the health risks of realistic doses of herbicides is obviously "influential" information as the Guidelines define the word; because it has a "clear and substantial impact on public policies or private sector decisions[, i.e. an impact] ...that has a high probability of occurring." The Guidelines fail to define "clear and substantial impact" as an impact that is important to the decision or policy being determined. That these EIS' separately evaluate the chronic health effects of herbicides shows that this information has a "clear and substantial" impact on their purpose, noxious weed control; therefore it is influential information.

As "influential information", the DQA Guidelines oblige the Forest Service to ensure that it is presented in a manner transparent and informative enough that the result could be replicated by an independent tester. Since many of the Forest Service's presented chronic toxicity results (derived from registration tests) have been refuted by published and peer reviewed data, it is non-objective data that must be removed from dissemination and corrected.

Presentation of *influential* scientific research information must also conform to the following criteria, when it pertains to risks to human health, safety and the environment:

- it must follow the risk assessment procedures contained in the Safe Drinking Water Act (SDWA);
- ensure that the presentation of information about risk is comprehensive;
- specify to the extent practicable each significant uncertainty; and
- specify additional studies (including peer-reviewed) known to the agency that speak to the issue.

The SDWA requires that the EPA conduct a hazard analysis that looks for literature pertaining to the risk being assessed. Obviously these FEIS's failed to perform such a literature search (even indirectly, by citing the conclusions of previous herbicide risk assessments performed for the USFS)--because if they had, they would have presented at least some of the peer reviewed published and government agency literature that we present now (and there's more that we have not come across); data which reaches the opposite conclusions the Forest Service reached.

UNCERTAINTIES (Data Gaps, or DQA 'Completeness')

The DQA Guidelines call for agencies to present information comprehensively and completely. These three Forest Service FEISs (and the herbicide risk assessments that they rely on) fail entirely to analyze the impact of the uncertainties created by relying on the registration's tests, which have many data gaps. When the FEIS acknowledge the failure of registration to test for a toxic endpoint they simply state that the infrequent use and the use mitigation measures will prevent any significant toxicity; or if they discuss the data gap, they completely fail to cosider the objective information we present in our appendix. Under NEPA too, an FEIS must evaluate these risks in order to evaluate their impact.

Entire life stages, systems and organs go untested by the registration tests relied on by the Forest Service! Moreover, for the majority of representative species no endpoint other than acute lethality is tested in pesticide registration. Much of the USFS' ecologic research focuses on aquatic ecosystems. Therefore there does exists some independent literature on pesticides' non-lethal acute effects for aquatic species--the area with the most complete toxicity tests of pesticides. Yet even here, these three FEISs rely on acute lethality (occasionally on non-lethal acute tests) of their herbicides to predict their chronic toxicity. They divide the lowest known acute adverse effect dose by a safety factor (such as 1,000) to calculate a supposedly completely safe concentration of the herbicide in water. The FEIS's show no relationship between acute and chronic toxicity. None of these FEISs nor the risks assessments that they cite evaluate the available and missing data for non-lethal and chronic toxicity tests for all non-mammalian species. Thus they fail your DQA Guidelines for treating influential information.

When chronic toxicity tests do exist, they typically use only somewhat lower doses than the acute tests do. Therefore the chronic toxicity tests don't test the effects of the actual doses that humans and other species typically receive. The three FEISs emphasize that there will not be repeated herbicide applications in most areas--they fail to search for literature on low dose herbicide toxicity. In recent years independent academics have begun to investigate the toxicity of real-world (lower) exposure levels. Their initial findings (see our appendix for a summary, in the area of pesticides alone) indicate that low dose toxicity is common. Such toxicity at ubiquitous doses--whether data gaps or what the independent literature shows--should have been analyzed under the DQA Guidelines for influential information.

The FEISs state that the actual exposures that will occur are mixtures. Even exposure from a single "pesticide" application is actually an exposure to the mix of chemicals in the pesticide formulation. But most of the tests required for pesticide registration do not include the so-called inert ingredients. While the three FEIS's discuss the exposure and known toxicity of some common 'inerts', they do not summarize the independent literature on the toxicology of herbicide products and mixtures. Our appendix's summary of that

literature for herbicides alone shows additivity of health effects at low doses of mixtures; and outright synergism of toxic effects of herbicide mixtures, at both high and low doses.

Finally, while the three FEIS's do consider herbicide drift more completely than they consider chronic toxicity, they fail to show that they adequately considered objective data such as that which we present in our appendix, even though usually much of this data was presented at the DEIS stage (as was most of the appendix's data on chronic toxicity).

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(Explanation of the Effect of the Alleged Error)

These EIS's are required to be written and released to the public by NEPA and the Administrative Procedures Act (APA) so that the USFS accurately and thoroughly evaluates the environmental impacts of its projects, with input from the public.

The non-objective data and data gaps used by the Forest Service causes a failure to evaluate impacts such as inadequate protection of the ecosystems that the USFS must maintain. Human and non-human residents and users in these three Forests rely heavily on the objectivity (accurate, unbiased and complete) of the FEIS's assessment of the risks of the herbicide spraying. Thus they may be significantly harmed by the lack of objective information of these assessments.

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(Recommendation and Justification for How the Information Should Be Corrected)

Because of the high environmental impact of this toxicology information, we now request that you suspend these three Forests' noxious weed control programs while the Forests adequately evaluate the non-lethal risks of the herbicides, under the DQA and NEPA.

The Forests must also re-evaluate the risks and efficacy of the various alternative weed control methods that were initially considered by these FEIS's, including the introduction and implantation of weed seeds by off-road recreation vehicles and other human activties. The FEIS's must require that activities that encourage weed problems are modified or eliminated. We also recommend that these FEIS's emphasize Integrated Weed Management (IWM). Currently IWM is largely un-implemented: only minimal acreage is treated w/ alternative methods, there is no gathering of data on the ecology and environment of the targeted weeds, and prevention efforts are weak. Last, the more that any single eradication control method is used--such as an herbicide, the more rapidly and strongly a species adapts to it. We present you with a summary of the independently-published data, which shows that herbicide resistance has grown exponentially with herbicide use.

Finally, we recommend that the USFS adopt this approach to all its work on controlling invasive species. That approach adequately assesses the large DQA-compliant database of independently published toxicity literature on pesticides; while it shifts a narrow reliance on pesticides to the multi-pronged, knowledge-based strategy of Integrated Pest Management (IPM), whose first tactic is always prevention.

In addition to these three FEIS, at least four NF in Montana alone have begun the NEPA process for similar Forest-wide noxious weed plans (the Helena, Gallatin, Kootenai and Lolo NF--the latter two being in the scoping stage and the other two awaiting finalization of their DEIS). These two DEIS show the same deficiencies as the three FEIS: a completely wrong summary table of chronic toxicity, and hardly any other supporting information. The USFS should correct such information deficiencies in all its work that involves pesticides.

Thank you for your careful consideration of this formal Request for Correction of Information, pursuant to the USFS's Information Quality Guidelines as required by the DQA.

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Sincerely,

Tony Tweedale, for:
The Alliance for the Wild Rockies
P.O. Box 8731
Missoula, MT 59807

Jeff Juel
The Ecology Center, Inc.
801 Sherwood, Suite D
Missoula, MT 59802

Jim Miller, President
Friend of the Bitterroot
P.O. Box 442
Hamilton, MT 59840

Katie Fite
Committee for the High Desert
PO Box 2863
Boise ID 83701

Alexandra Gorman, Director of Science and Research
Women's Voices for the Earth
P.O. Box 8743
Missoula, MT 59807

Caroline Cox, Staff Scientist
Northwest Coalition for Alternatives to Pesticides (NCAP)
PO Box 1393 Eugene OR 97440

Appendix follows.
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APPENDIX TO AWR et al.'s REQUEST FOR CORRECTION OF INFORMATION
(note: a complete reference may follow, not preceed, a brief (repeated) reference; because this is reorganized information orignially compiled elsewhere).

Contents:

- Critique of USFS "low/infrequent exposure equals safety" claim

- Critique of toxicity of mixtures USFS claims

- Critique of USFS pesticide drift claims

- Critique of the USFS summary chronic tox. tables

Critique of USFS "low/infrequent exposure equals safety" claim

Among herbicides alone, 60% are already documented to be hormone disruptors (without any hormone screening program--likely almost all chemicals are hormone disruptors).[1] 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.[2] 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.[3] Uncontrolled cellular division is called hyperplasia, a necessary step for cancerous tumors. In fact, prostate cancer is epidemic in industrialized countries.[4]

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.[5]

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.[6]

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.[7] Obesity rates are well-known to have exploded in recent decades in industrial countries,

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

[2] 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.

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

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

[5] 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.

[6] 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.

[7] 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.

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 invalid[8])

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.[9] 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.[10] 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.[11] 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.[12]

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.[13]

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).[14]

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[8] Welshons et al. 2003.

[9] T. Hayes et al. 2002 'Hermaphroditic, Demasculized Frogs After Exposure to the Herbicide Atrazine at Low Ecologically Relevant Doses' Proceedings Nti. 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.

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

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

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

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

[14] Sara Storrs and J Kiessecker July 2004 'Survivorship Patterns of Larval Amphibians Exposed to Low

Critique of toxicity of mixtures USFS claims

Without even a specific literature search, we know of these mixture toxicities *in the very narrow chemical catogory of herbicides*, ***alone*** (some clearly are examples of 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).[15]

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.[16]

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, but workers actually handle 2,4-D plus added chemicals.[17]

Older women exposed to triazine herbicides and thiocarbamate insecticides had eight times the rate of spontaneous absorption as those exposed to triazines only.[18]

Atrazine and alachlor herbicides together are acutely toxic to amphibian larvae.[19]

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.[20] (atrazine itself may contribute to the amphibian decline, given its ability to disrupt their sexual development at environmental concentrations[21]). 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;[22] 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 together they caused less chromosone damage than the N-NAt metabolite.[23]

Atrazine and metachlor (only when dosed together) delay metamorphosis of tadpoles to frogs by 10

Concentrations of Atrazine' Env. Health Perspectives:112:10:1054-7.

[15] Cavieres et al. 2002

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

[17] 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).

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

[19] Howe et al. 1998 'Effect of Chem. Synergy...' Env Toxicol Chem:17:519-525.

[20] 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.

[21] Hayes et al 2003.

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

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

days, at concentrations found in farm run-off.[24]

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).[25]

A mix of 2,4-D, glyphosate and triclopyr herbicides was toxic to larvae at levels lower than recommended for field applications.[26]

Picloram plus 2,4-D formulations (Tordon 202c) are a potent birth defect combination of a.i.[27]

While neither of these two a.i. alone damages the gill cells of catfish, in one of these Tordon formulations they do, an outright proof of synergy.[28]

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.[29]

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.[30] Early studies of this 'Agent White' mix looked at egg-hatching success;[31] and a more recent study examined the acute aquatic toxicity of 2,4-D, triclopyr and glyphosate mixes.[32]

Combinations of the herbicides alachlor, atrazine and picloram had various synergistic effects.[33]

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

[25] 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.

[26] Abdelghani et al. 1997 'Toxicol. Evaluation of Single & Chem. Mixtures..' Env. Toxicol. & Water Quality:12:237-48.

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

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

[29] 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.

[30] 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.

[31] 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.

[32] 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 & triclopyr. Bull Env Contam Toxicol 47:471-8.

[33] Information Ventures Inc. 1999 'Review of the Literature in Herbicides...IV. Health Effects of Other Herbicides'

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.[34]

Atrazine + methyl-parathion + methoxychlor was marginally synergistic (more than additive). Atrazine in binary combinations with other organophosphates indicate more than additive toxicity for all compounds except mevinophos.[35]

Later exposures to any toxin are made more dangerous by 2,4-D's de-activation of detoxifying liver enzymes.[36] 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.[37]

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.[38]

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 exposure levels.[39]

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.[40]

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.[41]

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.[42]

In the temporal and geographic vicinity of organophosphate insecticides, the unbelievably potent ALS inhibiting herbicides (the sulfonureas and the imidazaloninones) are even more (synergism) potent.[43]

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.[44]

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avail. at http://infoventures.com/e-hlth/ .

[34] W. Porter et al. 1999.

[35] Papelindstrom PA, Lydy MJ. 1997 Nov. Synergistic toxicity of atrazine and organophosphate insecticides contravenes the response addition mixture model. Environmental Toxicology & Chemistry 16:2415-2420.

[36] Rachel Carson 1962 'Silent Spring' Houghton Mifflin (Chapters 13-15).

[37] 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 nttp://www.ipm.iastate.edu/ipm/icm/2000/5-22-2000/interaction.html (accessed early 2004).

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

[39] 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.

[40] R. Blaustein, P. Johnson 'Explaining Frog Deformities' Feb. 2003 Sci. American, p. 60-5.

[41] 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.

[42] ATSDR 2002 'HSEES 1999-2000 Biennial Report', Atlanta GA (see Appen. C).

[43] R. Hartzler 2000.

[44] 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).

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.[45]

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.[46]

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.[47]

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.[48]

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 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.[49]

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.[50]

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.[51] 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.[52]

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

[46] 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.

[47] 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.

[48] 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.

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

[50] 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.

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

[52] 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.

Critique of USFS pesticide drift claims

EXTENT OF DRIFT, ESPECIALLY FROM AERIAL APPLICATION:

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."[53]

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).[54]

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.[55]

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.[56] 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).[57] Typically, 1% of an application reaches the direct target pest (e.g., weed), while 40% leaves the general target area.[58] Another estimate is that less than 0.1% of pesticides ever reach their target pests.[59] Also, it has been calculated that 20% of a pesticide application on surfaces volatilizes.[60] A 20 micron drop released just 10 feet off the ground will drift 1,056 feet in just a 3 mph breeze.[61] 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.[62] 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

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53 OH State U. Cooperative Extension Service 1992 'Reducing Spray Drift' Extension Bulletin #816; pg. 5.

54 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.

55 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

56 Teschke et al. Jan. 2001 'Spatial & Temporal Distribution of Airborne Bacillus thurungentius...' Env. Health Perspectives:109:47-52.

57 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.

58 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.

59 D. Pimentel April 1999. Speech at NCAMP/Beyond Pesticidesannual conference, Boulder CO (Dr. Pimentel is a noted researcher on pesticide economics and resistance at Cornell Univ.).

60 Dr. Alan Cessna , Env. Canada/Ntl. Water Research Institute Winter 2001 letter to the J. of Pesticide Reform.

61 Ohio Cooperative Extension Services Bulletin # 816, on pesticide drift.

62 S. Bird et al 1996 'Off-Target Deposition of Pesticides From Agricultural Aerial Spray Application' J. Env. Quality:25:5:1095-1104.

increased drift; drift nontheless increased by 29%.[63]

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.[64] 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[65] (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.[66] **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.[67] At least three more studies document significant 2,4-D (and triallate) drift beyond target areas.[68] Rainwater concetrations of 2,4-D have exceeded allowable contaminant levels.[69] 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.[70] 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.[71] 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.[72] 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).

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[63] 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.

[64] S. Bird et al 2002 'Evaluation of the AgDisp Aerial Spray Algorithms in the AgDrift Model' Env. Toxicol. & Chem.:21:3:672-681.

[65] M. Teske et al 2002 'AgDrift: A Model For Estimating Near-Field SPray Drift From Aerial APplications' Env. Toxicol. & Chem.:21:3:659-671

[66] C. Cox Spring 1995 'Indiscriminately From the Skies' J. Pesticide Reform:15:1:2-7 (citations #33-38).

[67] 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.

[68] 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.

[69] 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].

[70] A. Maciorowski 1994.

[71] Teschke et al. Jan. 2001.

[72] 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.

EFFECTS OF DRIFT AND VOLATIZATION:

Before describing the literature that consiusively 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.

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. California does attempt 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).[73] More recently (1998-2000) drift accounted for 51% of injuries, possibly due in part to more & improved reporting to the system.[74] Obviously these are almost all acute injuries, only. 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.[75] 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.[76] Other documentations of human injury from pesticide drift exist,[77] including from herbicides (2,4-D and paraquat, both applications resulting in severe chronic injury).[78] 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.[79] 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.[80]

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."[81] 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.

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.[82] In fact, following early (1989) and persistent

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[73] 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.

[74] M. Reeves et al. 2003 'Greater Risks, Fewer Rights: U.S. Farmworkers & Pesticides' Int'l J. Occup. & env. Health:9:30-39

[75] R. Fenske et al. 2000 'Strategies for Assessing Children's Organophophorous Pesticide Exposure in Agricultural Communities' J. Exposure Analysis & Env. Epidemiology:10:662-671.

[76] PANNA 2003.

[77] C. Cox Spring 1995 'Indiscriminately...' (citations #2-8, 50-51).

[78] C. Cox Spring 1995 'Indiscriminately...' (citations #4, 6 & 7).

[79] 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).

[80] A. DePalma 1 Sept. 2004 'When Pest Killers Make Mistakes' NYT, front pg. Metro sec.

[81] J. Barry et al. 1993 'Predicting and Measuring Drift of Bacillus thuringiensis Sprays' Env. Toxicol. & Chemistry:12:1977-1989.

[82] 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

problems in eastern WA state of crop damage from the long-range use of SU and other herbicides,[83] 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![84] Low chlorsulfuron 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.[85] Similar damage occured at even more tenous simulated SU drift (down to 0.33% of label application rate of a thifensulfuron/tribenuron mixed formula).[86] 1/500th the application rate of chlorsulfuron reduced cherry yield 85% w/out visible damage; and *1/10,000* of its label rate damaged white mustard seed production![87]

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.[88] 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).[89] Sub-lethal applications of 2,4-D and dicamaba caused persistant crop loss in experiments.[90] 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.[91] 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)[92] 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 species for MCPA and mecoprop, and asulam was the least toxic (they modeled that vaporization after glyphosate spray should not cause that damage, implying that drift would).[93]

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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.

83 O'Neal G. 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].

84 A. Maciorowski 1994.

85 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.

86 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.

87 A. Maciorowski 1994.

88 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).

89 M. Berrill et al. 1998 'Toxicity of Endosulfan to Aquatic Stages of Anuran Amphibians' Env. Toxicol. & Chem.:17:9:1738-1744.

90 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.

91 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).

92 Al-Khatib K, Mink GI, Reisenauer G, Parker R, Westberg H, Lamb B. 1998 '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.

93 V. Breeze et al. 1992 Dec. 'Use of a model and toxicity data to predict the risks to some wild plant species from drift

Critique of the USFS summary chronic tox. tables

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.[94] 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.[95] The exposure to HCB of ground applicators of picloram to exceed EPA's acceptable cancer risk level by ten-fold.[96] 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,[97] while male rats suffered atrophied testicles.[98] Picloram plus 2,4-D ('Tordon 202c' brand) is a very potent teratogen when fed to parent test animals--even to the father alone.[99]

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,[100] after initially finding no atrophy; a result that the manufacturer Dow disputed. Dow did find increased miscarriages at picloram the higher test dose/s,[101] and the State of California found increased embryo loss for the potassium salt formulation of the picloram molecule.[102] 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.[103] The same two a.i. sold as the 4Tordon75D formulation are severely toxic to test animal testicles.[104]

FEIS Claim: "Mutagenic: Unlikely"

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of 4 herbicides' Ann Appl Biol;121:669-77.

[94] NTP 1997 Report TR-23, 1978; also the IARC picloram monograph.

[95] EPA/OPP 1996 Picloram RED.

[96] EPA/OPP 1996 Picloram RED.

[97] California Dpt. of Food & Agriculture Medical Toxicology Branch 1988 'Summary of Toxicological Data, Picloram' Sacramento CA.

[98] EPA/Office of Drinking Water (ODW) 1988 'Picloram Health Advisory' Wash. DC.

[99] Described in the subsection 'MIXTURE TOXICITY AND SYNERGY TOXICITY', immediately after the critiques of individual herbicide of the FEIS' Summary Table.

[100] M. Reuber 1981 'Carcinogenicity of Picloram' J. Toxicol. & Env. Health:7:2:207-222.

[101] EPA 1995 (Picloram RED).

[102] Calif. DF&A 1988.

[103] P. Blakley et al. 1989--3 papers.

[104] Oakes et al. 2002.

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.[105] Picloram twice again tested positive for mutagenicity in tests.[106]

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 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.[107]

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.[108]

FEIS Claim: "Reproductive: No Effects"

What the Literature Says: Triclopyr caused reproductive effects in tests on rabbits and mice species.[109] 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.[110]

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).[111]

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105 Calif. DF&A 1988.

106 Muhammed et al. 1993 Mutat. Res.:426:2:193-199; and Venkat et al. 1995 Environ. Mol. Mutagen.:25:1:67-76.

107 EPA/OPP 1996. 'Carcinogenicity Review for Triclopyr' Wash. DC.

108 EPA/OPP 1998 'RED, Triclopyr' Wash. DC.

109 EPA/OPP 1998 (triclopyr RED).

110 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).

111 EPA/OPP 1998 (triclopyr RED).

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.[112] 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.

FEIS Claim: "Teratogenic: No Effects"

What the Literature Says: hydrocephaly and "[multiple] skeletal abnormalities were evident at all dose levels tested."[113] 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.[114]

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.[115]

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.[116] 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"

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[112] 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.

[113] EPA/OPP 1991 '..(clopyralid): Review of Rabbit Teratology Study Submitted by the Registrant', internal memo from T. McMahon, Mar. 20.

[114] 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.

[115] EPA/OPP 1991 (clopyralid review memo). **Also:** C. Cox Winter 1998 Editorial J. Pesticide Reform:18:4:inside front cover.

[116] Personal communication 24 Dec. 2002, EPA/OPPTS FOIA response to Tony Tweedale, Missoula MT.

What the Literature Says: Evidence of carcinogenicity is equivocal.[117] Hexazinone tested negative for carcinogenicity except in mice at the 300 mg/kg b.w./day dose.[118]

FEIS Claims: "Teratogenic: Unlikely"

What the Literature Says: Maternal dosing of rats above 400 mg/kg b.w./d caused birth defects.[119] Some developmental effects also occurred at higher dosing levels.[120]

FEIS Claims: "Reproductive: Unlikely"

What the Literature Says: Reproductive effects occurred at the mid and high dose levels--hardly "unlikely".[121]

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".[122]

It is worth noting that hexazinone's RfD ("safe" dose) is 0.033 mg/kg b.w./day.[123] 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.[124] Sulfometuron methyl also caused smaller

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117 EPA/OPP 1994 'RED Hexazinone Facts Summary'.

118 Weed Society of America (WSA)1994 Herbicide Handbook 7th Ed.

119 WSA 1994.

120 EPA/OPP 1994 (Hexazinone RED Summary).

121 EPA/OPP 1994 (Hexazinone RED Summary).

122 Weed Soc. 1994.

123 USFS & Bonneville Power Admin. 1992 'Risk Assessment for Herbicide Use...'.

124 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 28 Feb. 1993 'Sulfometuron Methyl-Evaluation of two-generation,...', internal memo of R. Fricke to L. DeLuise, Wash. D.C.

litters in both rats and rabbits.[125] Similar effects occurred in the test of a SU drug).[126]

As to carcinogenicity and mutagenicity: although DuPont's mutagenicity tests on sulfometuron methyl were negative, sulfometuron methyl metabolizes to saccharin,[127] 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.[128] 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.[129]

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.[130] 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.[131] SUs can affect thyroid hormone production and balance.[132]

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.[133] 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.[134] 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).[135] 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.[136] 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.[137]

IMAZAPYR (ARSENAL, CHOPPER, ASSAULT...etc.) AND IMAZAPIC (PLATEAU, CADRE, ...)

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[125] EPA/OPP 28 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.

[126] Seyler et al. 1992 Reprod. Toxicol.:6:447-452.

[127] EPA/OPP 6 Sept. 1991 'Pesticide environmental fate one-line summary: sulfometuron methyl', Wash. D.C.

[128] IARC 1999 'N-Vinyl-2-pyrrolidone and polyvinyl pyrrolidone' IARC Monographs:71:1181.

[129] EPA/OPP 1983 Memo from A. Arce to R. Taylor of E. I. DuPont, 13 Oct. (document ID#353-UNR).

[130] 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).

[131] D. Juurlink et al. (2 Apr. 2003) 'Drug-Drug Interactions Among Elderly..' JAMA:289:13:1652-8 (refer's 24-30).

[132] R. Guazelli et al. 1968 Acta Diabetol. Latina:5:614-623; and: J. Hershman et al. 1968 J: Clinical Endocrin.:28:1605-1610.

[133] Short & Colburn 1999 _Toxicol. & Industrial Health_:15:240-275 (summarizing all this data).

[134] J. Fletcher, T. Pfleeger & H. Ratsch 1998 '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.

[135] Journal of Pesticide Reform Fall '96 16:3:10-11.

[136] Multinational Monitor Jul./Aug. '93, p.4.

[137] I. Heap 2002 (i.e. the Weed Science Soc. Amer., http://www.weedscience.org).

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.[138] 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.[139] 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.[140] The only other ingredient in imazapic formulations which EPA has disclosed so far[141] is crystaline silica, a potent known human carcinogen when inhaled[142] (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 tumors.[143]

Similarly for imazpic the registration test for reproductive/developmental effects showed increasing rate of undeveloped ribs in rabbits,[144] 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.[145]

In addition to acute toxicity to non-target plants, a variety of other impacts have been reported.[146] 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.[147] 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.[148] 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

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138 EPA/OPP 1991 'Peer Review of Imazapyr' Oct. 2. memo from W. Dykstra.

139 C. Cox 1996 'Imazapyr Herbicide Fact Sheet', J. Pesticide Reform 16:3:16-17.

140 EPA/OPP/HED 2001 'Imazapic: Report of the Haz ard Identification Review Cmtee Memo from W. Dykstra to W. Donovan'. Wash, DC May 3.

141 C. Cox 2003 'Imazapic Factsheet' J Pesticide Reform:23:3:10-14 (see p. 10-11, 'Inerts').

142 Int'l Agency for Research on Cancer (IARC) 1997 'Monograph 68:41; avail. at: http://cie.iarc.fr/htdocs/monogrpahs/vol68/silica.ht

143 EPA/OPP 1989, '90 & '91 (2 Data Evaluation Reports & a Peer Review, all for imazapyr, by EPA's W. Dykstra).

144 EPA/OPP/HED 2001.

145 EPA/OPP/HED 2001.

146 Cox 1996 (Imazapyr Fact Sheet).

147 Weed Society of America 1994 'Herbicide Handbook' 7th Ed.

148 USFS 2001 'LNF BGWR&BAWM FEIS'; see table IV-16, p. IV-50 & 51.

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).[149] In the temporal and geographic vicinity of organophosphate insecticides, these herbicides are even more (synergistically) potent.[150]

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,[151] 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.[152] The amine salt version of dicamba is contaminated with potent oxidative-damage carcinogens such as dimethyl nitrosamine.[153]

FEIS Claim: "Teratogenic: No Effects"

What the Literature Says: Several birth defects are caused by dicamba (and/or its potent contaminants) at low doses.[154]

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.[155] Mallard egg development is stunted.[156] The contaminant 2,7-DCDD (a dioxin) is also a reproductive toxicant, among its numerous potent effects.[157]

FEIS Claim: "Mutagenicity: No Effects"

What the Literature Says: Dicamba significantly increases DNA unwinding, unscheduled DNA synthesis and causes sister chromatid exchanges.[158] Four earlier studies also show it is mutagenic, including in

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[149] 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.:6:965-9; **also** J. Fletcher 1993 (using SUs).

[150] 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).

[151] K. Cantor 1992 Cancer Res. 52:2447-2455.

[152] J. Huff et al. 1991 Env. Health Perspectives 93:247-270.

[153] ?, Pure & Applied Chem. 52:499-526 1980 Int'l Union of P&AC (IUPAC).

[154] EPA/ODW 1968 'Dicamba Health Advisory' Wash. DC. Also Federal Registers 48:52:11,113-4 and 11,119-20)

[155] EPA/ODW 1988.

[156] Hoffman et al. 1984 Arch. Env. Contam. & Toxicol.:13:15-27.

[157] Khera & Ruddick 1973, Adv. Chem. Ser.:120:70-84.

[158] P. Perocco et al. 1990 Env. Mol. Mutag.15:131-135.

applicators.[159]

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.[160] One EPA OPP staff person termed Monsanto's data suspicious, given the need to protect health .[161] Since that suspicious dispute, glyphosate exposure has since been associated with non-Hodgkin's lymphoma, NHL, in a small non-significant case-control group.[162] 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.[163] Glyphosate is again associated with significantly elevated risk of the rare hairy cell leukemia.[164] A 2003 study confirmed the association of glyphosate exposure with increased incidence of non-Hodgkin's lymphoma.[165]Even an industry study found 'somewhat elevated' rates of various cancers in mice given glyphosate.[166] Roundup is a potent steroid hormone disrupter.[167] EPA warned the Drug Enforcement Agency in 1985 that glyphosate increases male animal kidney tumors, in a dose-dependent manner.[168] 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.[169]

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)."[170] A study of pregnant rats given glyphosate in their drinking water showed that this exposure caused changes in the activity of three

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159 Plewa et al. 1984; Ma 1984; Yoder et al 1973 **all in** Mut. Res. (188:233-245; 188:157-167; 21:325-330); **and** Puaztal 1986 Acta Botany Hung.:32:163-168.

160 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.

161 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.

162 Hardell & Eriksson 1999.

163 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).

164 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.

165 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.

166 K. Pavkov & J. Turnier1986 '2-Year Chronic Toxicity & Oncogenicity Dietery Study With SC-0224 in Mice'. Report # T-11813, Farmington: Stauffer Chemical Co.

167 Walsh et al 2000 Env. Health Perspectives:108:769-776.

168 Pesticide &Toxic Chemical News 14 Aug. '85, p.8.

169 Hardell & Eriksson 1999.

170 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.

enzymes in their fetuses--enzymes related to energy production were affected in the liver, heart, and brain.[171] 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.[172]

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.[173] Human father's use of glyphosate correlates with increased miscarriages and premature births in farm families.[174] 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.[175] A case report of frequent menstruation from a student using a track where glyphosate was sprayed.[176] 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.[177] In a study of female rabbits given glyphosate orally during pregnancies, glyphosate caused a "slight" decrease in fetal weight in all three treated groups.[178]

FEIS Claim: "Mutagenic: No Effects"

What the Literature Says: Mice injected with glyphosate and Roundup witnessed increased frequency of chromosome damage and DNA damage increased in bone marrow, liver, and kidney.[179] In fruit flies, Roundup and Pondmaster both increased the frequency of sex-linked, recessive lethal mutations, showing that the formulation is very mutagenic.[180] 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.[181] Glyphosate caused sister-chromatid exchanges in human lymphoid cells[182] Even in studies by the manufacturer, it caused a variety of chromosone aberations and gene mutations in mice lymphoid cells,[183] 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.[184]

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171 Daruich et al. 2001 'Effect of herbicide glyphosate on enzymatic activity in pregnant rats and their fetuses' Environ. Res./Sect. A 85:226-231.

172 Arbuckle et al. 2001.

173 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.

174 D. A. Savitz, 1997. American Journal of Epidemiology: 146:1025-103.

175 Arbuckle et al. 2001.

176 Barnard & Heauser in NCAA Sports Sciences Education Newsletter Vol. 2 Fall 1995.

177 J. of Pesticide Reform Fall '98, letters.

178 EPA/Off. of Toxic Substances 1980 'Glyphosate submission of rat teratology, rabbit teratology' Reg. #524-308.

179 C. Bolognesi et al. 1997 'Genotoxic activity of glyphosate and its technical formulation Roundup' J. Agricultural Food Chemicals 45:1957-1962.

180 P. Kale. et al. 1995. 'Mutagenicity testing of nine herbicides and pesticides currently used in agriculture' Environ. Mol. Mutagen. 25:148-158. Also Peluso et al. 1998 Environ. Mol. Mutagen. 31:55-59.

181 C. Bolognesi. et al. 1997.

182 N. Vigfusson & E. Vyse 1980 'The Effect of the Pesticides...and Roundup on Sister-Chromatid Exchanges in...' Mutag. Res.:79:53-57.

183 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.

184 Peluso M 1998 '32P-postlabeling detection of DNA adducts in mice treated with the herbicide Roundup' Environ Mol

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.[185] As NCAP notes, glyphosate has been shown to be toxic in every standard category of toxicology testing.[186]

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.[187] 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 been treated with those herbicides.[188]

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.[189] Embryo deaths and kidney & urogenital defects resulted in 2,4-D experiments.[190] Increased spontaneous abortions resulted in a 2,4-D experiment.[191] Supernumery ribs resulted from 2,4-D dosing.[192] 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.[193] 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.[194] 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.[195] 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

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Mutagen.:31:1:55-9.

185 NCAMP 1997 Technical Rpt. 12:2.

186 C. Cox Fall 1998 'Glyphosate Factsheet' J. of Pesticide Reform:18:3:3-16.

187 A.L. Schmidt 10 May 2003 'Poisonous Spray on Course Towards Drinking Water' Politiken, Denmark (avail.: http:politiken.dk/VisArtikle.sasp?PageID=269614).

188 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.

189 Reported in Trial, Nov. 1983 p. 97; and J. Schardein ed. 1983 Chemically Induced Birth Defects 2nd Ed., New York:Marcel Dekker.

190 D. Fofana et al. 2000 'Prenatal Developmental Effects of Pure 2,4-D Acid on the Rat' Congen.Anomal.:40:287-296.

191 T. Arbuckle et al. 1999 'Exposure to Chlorophenoxy Herbicides and the Risk of Spontaneous Abortions' Epidemiology:10:752-760.

192 N. Chernoff et al. 1990 'Effects of Chemically Induced Maternal Toxicity On Prenatal Development In the Rat' Teratology:42:651-658.

193 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.

194 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.

195 Garry et al. 1996.

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.[196] In traditional high dose (ppm) animal studies, 2,4-D caused bleeding of the abdominal cavity of rat fetuses.[197]

FEIS Claim: "Reproductive: Unlikely"

What the Literature Says: 2,4-D is found in the semen of agricultural workers.[198] Men with higher body burdens of 2,4-D had significant levels of semem abnormalities.[199] Sperm quality and quantity were severely affected in 32 farmers using 2,4-D (urinary verification);[200] corroborated by the Minnesota farmers teratogenicity study where in addition birth frequency was half that of non-exposed farmers, and leutenizing hormone levels were too high.[201] 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.[202] A single high dose of 2,4-D caused a 29% reduction in DNA synthesis in the testis of mice.[203] 2,4-D alters testicular Leydig cells.[204] High subchronic doses of 2,4-D caused testicular atropphy in rats.[205] Women using chlorophenoxy herbicides (and organophosphate insecticides) had significantly lower fecundity.[206] 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.[207] The same two a.i. sold as the Tordon75D formulation are severely toxic to test animal testicles.[208] 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).[209]

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196 Schreinemachers July 2003.

197 ExToxNet 1996 '2,4-D Pesticide Information Profile', Extension Toxciology Network; available http://extoxnet.orst.edu/pips/24-D.htm, accessed 30 July 2004.

198 T. Arbuckle et al. 1999 '2,4-D Acid Residues in Semen of Ontario Farmers' Reprod. Toxicol.:13:6:421-9.

199 Swan et al. Sept. 2003.

200 Lerda & Rizzi 1991 'Study of Reproductive Function in Persons Occupationally Exposed to 2,4-D Acid' Mut. Res. 262:47-50.

201 Garry et al. 1996.

202 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.

203 J. Seiler 1979 'Phenoxyacids As Inhibitors of Testicular DNA synthesis in Male Mice' Bull. Env. Contam. & Toxicol.:21:1&2:89-92.

204 R.C. Liu et al. 1996 'The Leydig Cell Function in Vitro' Fundam. & Applied Toxicol.:30:102-8.

205 J. Charles et al. 1996 'Comparative Subchronic Studies on 2,4-D Acid, Amine & Ester in Rats' Fundam. & Applied Toxicol.:33:2:161-5.

206 K. Curtis et al. 1999 'The Effect of Pesticide Exposure on Time to Pregnancy' Epidemiology 10:112-117.

207 P. Blakley et al. 1989--3 papers.

208 Oakes et al. 2002.

209 A. Greenlee et al. 2003. 'Risk factors for female infertility in an agricultural region' Epidemiology:14:429-436.

FEIS Claim: "Cancer: Unknown"

FEIS Claim: "Mutagenicity: Unlikely"

Below (to the end), 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 data below is a summary of *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). This *randomly-selected* complete literature search overwhelmingly finds that 2,4-D is carcinogenic and mutagenic; **thus there is no doubt** that complete literature searches for all 11 herbicides and their other chronic effects that the USFS evaluated would yield similar overwhelming findings of chronic toxicity. Neither the EPA nor we have removed the findings of no effect by self-interested parties that managed to get published (almost without exception in a journal with low peer-review standards); doing so would leave hardly any valid claims of no toxicity. Nor have we attempted to present to you any of the published literature on chronic effects *other than* those for which data is submitted during registration (the same four categories of chronic toxicity that the USFS has reviewed), such as endocrine, immunologic and neurologic toxicities. Note that mutagenicity (DNA damage) may lead to far more effects than cancer alone.

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POSITIVE CANCER RESULTS/GENERAL

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.

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:52:19:5485s-5488s). 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.
> Abstract: 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 isooctylester 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.

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 auxiliary and groin lymphatic nodes were seen in group VI in comparison with the control animals (group III).

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. **However, it does not significantly influence the growth of the carcinoma in the rats exposed only in the postlactational period**.

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 cPAD will be protective of the possible carcinogenic activity of this chemical."??

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 -0.6% 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: 14594619 [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 agricultural 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. 1999. Incidence rates of lymphomas and environmental measurements of phenoxy herbicides: ecological analysis and case-control study. Arch Environ Health 53:394-397.
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 1991, 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. 1991 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, 1.69; 95% CI, 1.32-2.69). 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, 1.69; 95% CI, 1.00-2.61); 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 661 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 = 360 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: 8016599 [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:
Stemberg 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, 3.52), 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 8.9 (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)

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 576 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 1.8) 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 1990-1989 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 1998 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):729-31. [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 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 1.90 [95% confidence interval (CI) = 1.02-3.19] 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: 529960 [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 159 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 = 1.1-29), 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]

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 45:96-105.
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 879 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. 1998. 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 dogs. One hundred one cases with cytologically or histologically confirmed lymphoma diagnosed at a veterinary teaching hospital between the middle of 1996 and the middle of 1999 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 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 age 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 dates 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 (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 two published results indicating that 2,4-D is not mutagenic. Considering quality, there is just one, as the other (published as three sequential papers) is authored by consultants paid by the 2,4-D industry, and published in a journal that accepts authors with horrible conflicts of interests. In contrast, we found 15 published papers showing that 2,4-D is mutagenic (all in journals with quality peer review).

Given that 19 of the 20 valid published studies of 2,4-D's mutagenicity prove that it is a mutagenic chemical, and that the weight of the evidence shows it is at least a possible human carcinogen, it is very important that you consider your own (one author) recent study finding that 13 of 18 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 55:339-343
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 14.92% 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:

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.

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 SUPER-LOW (5 PICOMOLE) 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:

Komuta 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, Jafal 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. 1992 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(0) 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:

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).

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.

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-G0 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. 1994. 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. 1994 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 those 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.

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 trisopropanolamine) 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. 1993. 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.

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 SUPER-LOW (5 PICOMOLE) DOSE EFFECT**]

Kaloumova 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. Z-VAD-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 lg/ml. The commerical-grade herbicide 2,4-D LV4 and 2,4-D amine induced cell proliferaton at 1 and 10 lg/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 4.1lg/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 acid 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:

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:

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 from 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:]

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 JC-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:

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 +/- 6.2% 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.

NEGATIVE RESULT/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.

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 B6C3F1 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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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.

POSITIVE RESULTS/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, Mamiemi 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: 6624479 [PubMed - indexed for MEDLINE]

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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 18% 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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