August 23, 2004

Environmental Protection Agency
Public Information and Records Integrity Branch (7502C)
Office of Pesticide Programs
1200 Pennsylvania Ave., NW
Washington, DC 20460-0001

RE: 2,4-D Risk Assessment; Docket ID No. OPP-2004-0167

Submitted electronically to opp-docket@epa.gov

Dear Sir or Madam:

We submit the following comments on behalf of Beyond Pesticides; Natural Resources Defense Council; TEDX, Inc. (The Endocrine Disruption Exchange); Pesticide Action Network North America; Northwest Coalition for Alternatives to Pesticides; Washington Toxics Coalition; Coalition for Health, Environment and Economic Rights; Cancer Prevention Coalition; The Breast Cancer Fund; Alliance for Healthy Homes; Farmworker Justice Fund, Inc.; Agricultural Resources Center; Institute for Agriculture and Trade Policy; Roseland Organic Farms; Safer Pest Control Project; Defenders of Wildlife; California Safe Schools; Advocates for Environmental Human Rights; Californians for Alternatives to Toxics; New York Public Interest Research Group; New Jersey Environmental Federation; Wyoming Outdoor Council; Alaska Community Action on Toxics; Ecology Center; Citizens' Environmental Coalition; Environmental Research Foundation; Clean Water Action; Toxics Action Center; Informed Choices; National Center for Environmental Health Strategies, Inc.; The Coalition for Alternatives to Pesticides; Texans for Alternatives to Pesticides; Jack B. Richman Environmental Coalition; Colorado Pesticide Network; Grassroots Coalition; Women's Voices for the Earth; Grassroots Environmental Education; No Spray Coalition, Nashville; Students for Bhopal; Citizens' Campaign for the Environment; Connecticut Coalition for

Environmental Justice; and the Coalition for Environmentally Safe Communities. (See page 33 to 36 for signatures.)

None of our organizations has any direct or indirect financial or fiduciary interest in the manufacture or sale of 2,4-dichlorophenoxyacetic acid, amine salts, esters, or any related alkylchlorophenoxy chemicals.

Unless otherwise noted or referenced, all page number references in the text refer to the following document, which is the focus of these comments: US EPA. 2,4-D. HED's Human Health Risk Assessment for the Reregistration Eligibility Decision (RED) Revised to Reflect Error-only Comments from Registrants. PC Code 030001; DP Barcode D293129.

SUMMARY OF COMMENTS

Our organizations have significant concerns about the EPA human health risk assessment for 2,4-D. In particular, we raise the following issues in these comments:

(1) EPA proposes to remove the full FQPA-mandated 10X safety factor to protect children from 2,4-D despite the evidence of their greater susceptibility to 2,4-D and the existence of significant data gaps related to both 2,4-D's toxicity and exposure;
(2) EPA does not designate farm children as a population of special concern and ignores aggregate risk to these children as a result oftheir additional pathways of exposure;
(3) EPA underestimates exposure risk and overestimates safety to farmworkers;
(4) EPA selects a dermal absorption factor that fails to protect infants, people wearing DEET or sunscreen, and people who have consumed alcohol, all of whom will have enhanced absorption of 2,4-D through the skin;
(5) EPA fails to fully assess risk to toddlers playing on lawns that were recently treated with 2,4-D;
(6) EPA fails to use the maximum water concentration of 2,4-D and because of the data gaps for dissipation rates, underestimates potential exposure risks
(7) EPA underestimates risks to swimmers in treated waters due to deficient reasoning and a failure to identify a data gap in dissipation rates;
(8) EPA fails to fully assess the risk of inhalation of 2,4-D;
(9) EPA ignores data showing low-dose toxicity in dogs without sufficient scientific justification;
(10) EPA fails to properly classify the carcinogenicity of 2,4-D in a Class C or B. The Agency ignores overwhelming and unique data showing that 2,4-D is cytotoxic, genotoxic, and has been associated with cancer in humans.;
(11) EPA fails to adequately address aggregate risk; and the
(12) EPA fails to meet its statutory obligation to assess combined effects.

INTRODUCTION

2,4-D (2,4-dichlorophenoxyacetic acid) is a common herbicide used around the home and garden, on golf courses, ball fields, parks, and in agriculture. This chemical is one of the first pesticides ever registered in the United States. Agricultural uses include pasture land, wheat, corn, soybeans, barley, rice, oats, and sugar cane. About 40 million pounds of 2,4-D are used in the U.S. every year. 2,4-D has a soil half-life of about one week. However, when tracked indoors and not exposed to direct sunlight, 2,4-D can be expected to persist in carpets for up to one year after a single turf application at a concentration of approximately 0.5 µg/g.[1] Despite a short half-life, the herbicide is found as a contaminant in surface water samples across the U.S., and has been detected in groundwater in at least five states and Canada[2].

Numerous epidemiological studies have linked 2,4-D to non-Hodgkin's lymphoma (NHL) among farmers.[3] Multi-center studies in Canada and in Sweden of members of the general public found a 30-50% higher odds of 2,4-D exposure among people with NHL.[4] [5] National Cancer Institute studies in household dogs have also reported an association between exposure to 2,4-D and canine malignant lymphoma.[6] A follow up study of the latter further strengthened the results.[7]

---

[1] Nishioka MG, Burkholder HM, Brinkman MC, Gordon SM. 1996. Measuring lawn transport of lawn-applied herbicide acids from turf to home: Correlation of dislodgeable 2,4-D turf residues with carpet dust and carpet surface residues. Environmental Science and Technology 30: 3313-3320.

[2] Extension Toxicology Network. 1996. Pesticide Information Profile for 2,4-D. http://extoxnet.orst.edu/pips/24-D.htm (Accessed June 21, 2004).

[3] Zahm SH. 1997. Mortality study of pesticide applicators and other employees of a lawn care service company. J Occup Environ Medicine 39: 1055-67; Fontana A, Picoco C, Masala G, Prastaro C, Vineis P. 1998. Incidence rates of lymphomas and environmental measurements of phenoxy herbicides: ecological analysis and case-control study. Arch Environ Health 53 :384-7; Zahm SH, Blair A. 1992Pesticides and non-Hodgkin's lymphoma. Cancer Res52: 5485s-5488s; Morrison HI, Wilkins K, Semenciw R, Mao Y, Wigle D. 1992. Herbicides and cancer. J Natl Cancer Inst 84:1866-74.

[4] McDuffie HH, Pahwa P, McLaughlin JR, et al. 2001. Non-Hodgkin's lymphoma and specific pesticide exposures in men: cross-Canada study of pesticides and health. Cancer Epidemiol Biomarkers Prev. 10(11): 1155-63.

[5] Hardell L, Eriksson M. 1999. A case-control study of non-Hodgkin lymphoma and exposure to pesticides. Cancer 85: 1353-60.

[6] 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(17): 1226-31.

[7] 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: 119-25.

2,4-D causes significant suppression of thyroid hormone levels in ewes dosed with this chemical.[8] Similar findings have been reported in rodents, with suppression of thyroid hormone levels, increases in thyroid gland weight, and decreases in weight of the ovaries and testes.[9] The increases in thyroid gland weight are consistent with the suppression of thyroid hormones, since the gland generally hypertrophies in an attempt to compensate for insufficient circulating levels of thyroid hormones. Thyroid hormone is known to play a critical role in the development of the brain. Slight thyroid suppression has been shown to adversely affect neurological development in the fetus, resulting in lasting effects on child learning and behavior.[10]

2,4-D causes slight decreases in testosterone release and significant increases in estrogen release from testicular cells.[11] In rodents, this chemical also increases levels of the hormones progesterone and prolactin, and causes abnormalities in the estrus cycle.[12] Male farm sprayers exposed to 2,4-D had lower sperm counts and more spermatic abnormalities compared to men who were not exposed to this chemical.[13] In Minnesota, higher rates of birth defects have been observed in areas of the state with the highest use of 2,4-D and other herbicides of the same class. This increase in birth defects was most pronounced among infants who were conceived in the spring, the time of greatest herbicide use.[14]

2,4-D also interferes with the neurotransmitters serotonin and dopamine. In young organisms, exposure to 2,4-D results in delays in brain development and abnormal behavior patterns, including apathy, decreased social interactions, repetitive movements,

---

[8] Rawlings NC, Cook SJ, Waldbillig D. 1998. Effects of the pesticides carbofuran, chlorpyrifos, dimethoate, lindane, triallate, trifluralin, 2,4-D, and pentachlorophenol on the metabolic endocrine and reproductive endocrine system in ewes. J Toxicol Environ Hlth 54:21-36.

[9] Charles JM, Cunny HC, Wilson RD, Bus JS. 1996. Comparative subchronic studies on 2,4-dichlorophenoxyacetic acid, amine, and ester in rats. Fundamental & Applied Toxicol 33: 161-165.

[10] Haddow JE, Palomaki GE, Allan WC, Williams JR, Knight GJ, Gagnon J, O'Heir CE, Mitchell ML, Hermos RJ, Waisbren SE, Faix JD, Klein RZ. 1999. Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child. New Eng J Med 341(8): 549-555.

[11] Liu RC, Hahn C, Hurtt ME. 1996. The direct effect of hepatic peroxisome proliferators on rat leydig cell function in vitro. Fundamental & Applied Toxicol 30:102-108.

[12] Duffard R, Bortolozzi A, Ferri A, Garcia G, Evangelista de Duffard AM. 1995. Developmental neurotoxicity of the herbicide 2,4-dichlorophenoxyacetic acid. Neurotoxicology 16(4):764.

[13] Lerda D, Rizzi R.1991. Study of reproductive function in persons occupationally exposed to 2,4-D. Mutation Research 262:47-50.

[14] Garry VF, Schreinemachers D, Harkins ME, et al. 1996. Pesticide appliers, biocides, and birth defects in rural Minnesota. Environ Hlth Perspect 104: 394-399.

tremor, and immobility.[15] Females are more severely affected than males. Rodent studies have revealed a region-specific neurotoxic effect on the basal ganglia of the brain, resulting in an array of effects on critical neurotransmitters and adverse effects on behavior.[16] A peer-reviewed, developmental neurotoxicity study demonstrated severe neurotoxicity in young rats exposed to 2,4-D from postnatal days 12 to 25 at doses of 70 mg/kg/day. These pups showed decreases in GM1 level, diminution in myelin deposition and alterations in all behavioral tests at all doses.[17] This herbicide specifically appears to impair normal deposition of myelin in the developing brain.[18] The neurotoxic and anti-thyroid effects of 2,4-D make it highly likely that fetuses, infants, and children will be more susceptible to long-term adverse health effects from exposure to this chemical although they may appear normal at birth.

Young animals can also be exposed to 2,4-D through maternal milk. Recent research has revealed that 2,4-D is excreted in breast milk, thereby resulting in potentially significant exposures to the nurseling. The researchers detected 2,4-D residues in stomach content, blood, brain and kidney of 4-day-old neonates fed by 2,4-D exposed mothers.[19] When maternal exposures stopped, the chemical continued to be excreted in maternal milk for a week. Thus, postnatal exposures to this chemical during the critical period for development of the infant brain are of serious scientific concern.

(1) EPA Must Use the FQPA-Mandated 10X Safety Factor to Protect Children

Under the Federal Food, Drug, and Cosmetic Act (FFDCA), as amended by the Food Quality Protection Act (FQPA), the Environmental Protection Agency (EPA) may only establish a tolerance for pesticide chemical residue in or on a food if EPA

---

[15] Evangelista de Duffard AM, Bortolozzi A, Duffard RO. 1995. Altered behavioral responses in 2,4-dichlorophenoxyacetic acid treated and amphetamine challenged rats. Neurotoxicology 16(3): 479-488.
[16] Bortolozzi A, Evangelista de Duffard AM, Dajas F, Duffard R, Silveira R. 2001. Intracerebral administration of 2,4-diclorophenoxyacetic acid induces behavioral and neurochemical alterations in the rat brain. Neurotoxicology 22(2):221-32.
[17] Rosso SB, Garcia GB, Madariaga MJ, Evangelista de Duffard AM, Duffard RO. 2000. 2,4-Dichlorophenoxyacetic acid in developing rats alters behaviour, myelination and regions brain gangliosides pattern. Neurotoxicology 21(1-2):155-63.
[18]. Duffard R, Garcia G, Rosso S, Bortolozzi A, Madariaga M, di Paolo O, Evangelista de Duffard AM. 1996. Central nervous system myelin deficit in rats exposed to 2,4-dichlorophenoxyacetic acid throughout lactation. Neurotoxicol Teratol 18(6): 691-6.
[19]. Sturtz N, Evangelista de Duffard AM, Duffard R. 2000. Detection of 2,4-dichlorophenoxyacetic acid (2,4-D) residues in neonates breast-fed by 2,4-D exposed dams. Neurotoxicology 21(1-2): 147-54.

determines that the tolerance is "safe." 21 U.S.C. § 346a(b)(2)(A)(i). A tolerance will meet this requirement only if "there is a reasonable certainty that no harm will result from aggregate exposure to the pesticide chemical residue, including all anticipated dietary exposures and all other exposures for which there is reliable information." *Id.* § 346a(b)(2)(A)(ii). The health-protective standard of the FQPA requires EPA to give special consideration to the health of infants and children, and EPA must "ensure that there is a reasonable certainty that no harm will result to infants and children from aggregate exposure to the pesticide chemical residue." *Id.* § 346a(b)(2)(C)(ii)(i). In the Revised Human Health Risk Assessment, EPA fails to ensure a "reasonable certainty that no harm will result to infants and children"

In particular, EPA fails to include an additional 10X safety factor for infants and children as required by the FQPA. Under the Food Quality Protection Act's precautionary approach to protecting children, EPA must maintain an additional 10-fold margin of safety in its risk assessments for individual pesticides to "take into account potential pre– and post–natal developmental toxicity and completeness of the data with respect to exposure and toxicity to infants and children." 21 U.S.C. § 346a(b)(2)(C). EPA can use a different margin of safety "only if, on the basis of reliable data, such margin will be safe for infants and children." *Id.* Yet there are significant toxicity and exposure data gaps acknowledged by EPA in the current risk assessment. In particular, EPA has acknowledged that it lacks required data to assess toxicity to the developing brain and nervous system (p.35), adequate data to assess toxicity to the endocrine system (p.40), adequate data to fully assess reproductive toxicity (p.28) and data to assess inhalation risk from 2,4-D (p.29). Therefore, the Agency lacks the "reliable data" necessary under the FQPA to authorize the removal of the 10X safety factor or a different margin of safety.

On page 8 of the "HED's Human Health Risk Assessment for the Reregistration Eligibility Decision (RED) Revised to Reflect Error-only Comments from Registrants," EPA concludes: "At the 4/8/03 HIARC meeting, it was determined that, based on the 2,4-D database summarized above, no special FQPA Safety Factor is needed [1X] since there are no residual uncertainties for pre- and/or postnatal toxicity…."

This conclusion is based on incomplete consideration of the evidence. The Agency's conclusion that there is no need for the FQPA Safety Factor since there are no residual uncertainties for pre-and/or postnatal toxicity is based upon the assumption that the exposure databases are complete. The EPA assessment of developmental toxicity used two studies of prenatal development that did not show reduced survival, one of which showed a LOAEL of 75 mg/kg/day in the rat (Table 4). However, the Agency did not consider at least one recent study that did find reduced survival at lower levels of exposure.[20] Another key study that the Agency has yet to consider revealed the insidious nature of 2,4-D. Exposure was perinatal**,** but the damage was not expressed until adulthood.[21]

Furthermore, as the Agency is well aware, data on endocrine disruption is extremely critical to children's health and the health of the general population and wildlife. The Agency was mandated by FQPA to establish a protocol for reviewing endocrine disruption within 2 years of its passage in 1996. In this risk assessment, the Agency states, "When the appropriate screening and/or testing protocols being considered under the Agency's EDSP have been developed, 2,4-D may be subjected to additional screening and/or testing to better characterize effects related to endocrine disruption." (p.40) As the Agency is still obligated under several different statutes to protect the health of infants and children; and as all the current data using high-dose levels are not applicable for endocrine disruptors; and as endocrine disruption concerns timing of exposure and low dose levels; and until the Agency establishes a protocol and proves differently; it has a legal obligation to automatically retain the 10X safety factor for children. This is further supported by the peer-reviewed literature that acknowledges the weight-of-evidence of 2,4-D as a likely endocrine disruptor. (See Introduction.)

While we applaud the agency for requiring a developmental neurotoxicity (DNT) study in the rat, it must be recognized that (a) the very absence of this study by itself should prohibit EPA from overturning the default 10X safety factor, and (b) the study alone will not suffice to remove the uncertainty around neurotoxicity. In its 1993 report,

---

[20] Fofana D, Kobae H, Sameshima K, Miyata K. 2002 Mar. Postnatal survival of rat offspring prenatally exposed to pure 2,4-dichlorophenoxyacetic acid (2,4-D). Congenit Anom (Kyoto) 42:32-5.

[21] 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.

Pesticides in the Diets of Infants and Children, the National Academy of Sciences/National Research Council cited strong evidence that pesticide exposures may disrupt the normal development of a child's brain and nervous system. More conclusive evidence has since been published supporting this finding.[22] Studies by EPA staff scientist Dr. Makris show that DNT testing is more sensitive than other studies in measuring the effects of exposure on proper development of the brain and nervous system, and therefore DNT testing is more appropriate for protecting children's health. DNT testing is essential for pesticides, not only as a measure of toxicity to the developing brain and nervous system, but also as an often more sensitive measure of developmental and reproductive effects generally.[23] EPA's 10X Task Force has recommended that "developmental neurotoxicity testing be included as part of the minimum core toxicology data set for all chemical food-use pesticides for which a tolerance would be set."[24] Although DNT testing has not yet been incorporated in the minimum core toxicology data set for all pesticides, EPA has required DNT studies on a case-by-case basis for particular pesticides, including 2,4-D. 67 Fed. Reg. 10627.

We support EPA's decision to add an additional 10-fold "database uncertainty factor" to account for the serious gaps in the available toxicology data for 2,4-D, including the absence of the DNT study, the absence of adequate testing for endocrine disruption, the deficiencies in the immunotoxicity data, and the absence of an inhalation toxicity study. However, the use of a database uncertainty factor in no way absolves EPA

---

[22] Crumpton TL, Seidler FJ, Slotkin TA. 2000. Developmental neurotoxicity of chlorpyrifos in vivo and in vitro: effects on nuclear transcription factors involved in cell replication and differentiation. Brain Res 857: 87-98; Dam K, Seidler FJ, Slotkin TA. 1998. Developmental neurotoxicity of chlorpyrifos: delayed targeting of DNA synthesis after repeated administration. Brain Res Dev Brain Res 108: 39-45; Dam K, Seidler FJ, Slotkin TA. 1999. Chlorpyrifos releases norepinephrine from adult and neonatal rat brain synaptosomes. Brain Res Dev Brain Res 118: 129-33; Dam K, Garcia SJ, Seidler FJ, Slotkin TA. 1999. Neonatal chlorpyrifos exposure alters synaptic development and neuronal activity in cholinergic and catecholaminergic pathways. Brain Res Dev Brain Res 116: 9-20; Dam K, Seidler FJ, Slotkin TA. 2000. Chlorpyrifos exposure during a critical neonatal period elicits gender- selective deficits in the development of coordination skills and locomotor activity. Brain Res Dev Brain Res 121:179-87; Levin ED, Addy N, Nakajima A, Christopher NC, Seidler FJ, Slotkin TA. 2001. Persistent behavioral consequences of neonatal chlorpyrifos exposure in rats. Brain Res Dev Brain Res 130:83-9; Raines KW, Seidler FJ, Slotkin TA. 2001. Alterations in serotonin transporter expression in brain regions of rats exposed neonatally to chlorpyrifos. Brain Res Dev Brain Res 130:65-72.

[23] Kimmel CA, Makris SL. 2001. Recent developments in regulatory requirements for developmental toxicology. Toxicol Lett 120:73-82.

[24] 10X Task Force, U.S. Environmental Protection Agency, *Toxicology Data Requirements for Assessing Risks of Pesticide Exposure to Children's Health (draft)*, Nov. 30, 1998, 11.

of its statutory responsibility to use the FQPA-mandated child-protective safety factor. Therefore both the database uncertainty factor and the child-protective safety factor must be used.

Had EPA not removed the child-protective 10X safety factor, 2,4-D would have still had to classify most human exposures to 2,4-D as unsafe. Even ignoring all of the other flaws addressed below in EPA's risk assessment for this pesticide, this single decision to overturn 10X resulted in unsafe tolerances improperly being declared "safe."

In light of the existing evidence that 2,4-D is especially toxic to the developing organism, EPA's failure to apply the 10X children's safety factor in setting tolerances most egregiously violates the FQPA and EPA's own stated policy on proper application of the 10X safety factor. "Risk assessors . . . should presume that the default 10X safety factor applies and should only recommend a different factor, based on an individualized assessment, when reliable data show that such a different factor is safe for infants and children."[25]) For example, as discussed below, EPA assesses the acute risk to toddlers based on the acute neurotoxic effects in the adult, without adjusting the required margin of safety, even though the required developmental neurotoxicity study has not yet been done.

(2) Farm children are especially vulnerable to pesticide exposure, yet their risks are not assessed by EPA

Farm children are an especially vulnerable population, and their exposure to 2,4-D must be considered in assessing human health risk from pesticides. The FQPA requires that EPA consider exposure not just to consumers as a whole, but also to "major identifiable subgroups of consumers." 21 U.S.C. § 346a(b)(2)(D). In establishing tolerances, EPA must consider, among other relevant factors, "available information concerning the dietary consumption patterns of consumers (and major identifiable subgroups of consumers); . . . available information concerning the aggregate exposure levels of consumers (and major identifiable subgroups of consumers);" and "available information concerning the variability of the sensitivities of major identifiable subgroups

[25] (Office of Pesticide Programs, U.S. Environmental Protection Agency, *Determination of the Appropriate FQPA Safety Factor(s) in Tolerance Assessment*, Feb. 28, 2002, at 11.

of consumers." 21 U.S.C. § 346a(b)(2)(D)(iv); (vi); (vii). Farm children are a major identifiable subgroup under these statutory provisions, and their unique dietary consumption patterns, aggregate exposure levels, and sensitivities to exposure should have been assessed by EPA in establishing new tolerances for 2,4-D.

More than 320,000 children under the age of six live on farms in the United States. In addition, many hundreds of thousands of children play or attend schools on or near agricultural land, and others have family members who work on farms or handle pesticides as part of their jobs. The nation's 2.5 million farm workers have approximately one million children living in the United States.[26] .

Children living in agricultural communities are heavily exposed to pesticides, whether or not they work in the fields.[27] Farm children come in contact with pesticides through residues from their parents' clothing, dust tracked into their homes, contaminated soil in areas where they play, food eaten directly from the fields, drift from aerial spraying, contaminated well water, and breast milk. Furthermore, farm children often accompany their parents to work in the fields, raising their pesticide exposures even higher.[28] Citing data from the Department of Labor, the U.S. General Accounting Office has reported that seven percent of farmworkers with children five years old or younger took their children with them when they worked in the fields.[29] Children age nine or older may and do work on large farms. Farm children are likely to have the highest exposure to pesticides of any group of people in the country. Many of the children with the greatest pesticide exposures are from migrant farmworker families.[30] A study of migrant children in western New York found that despite legal prohibitions against working with hazardous substances, 10% of children under age 18 reported mixing or

---

[26] NRDC et al., *Petition for a Directive that the Agency Designate Farm Children As a Major Identifiable Subgroup and Population at Special Risk to be Protected under the Food Quality Protection Act*, Oct. 22, 1998, at 1 (hereafter "NRDC, *Farm Kids Petition"*).

[27] Lu C, Fenske RA, Simcox NJ, Kalman D. 2000. Pesticide exposure of children in an agricultural community: evidence of household proximity to farmland and take home exposure pathways. Environ Res 84:290-302; Loewenherz C, Fenske RA, Simcox NJ, Bellamy G, Kalman D. 1997. Biological monitoring of organophosphorus pesticide exposure among children of agricultural workers in central Washington State. Environ Health Perspect 105:1344-53; Fenske RA. 1997. Pesticide exposure assessment of workers and their families. Occup Med 12:221-37.

[28] NRDC, *Farm Kids Petition*, at 2-3.

[29] U.S. General Accounting Office, *Pesticides: Improvements Needed to Ensure the Safety of Farmworkers and Their Children*, (RCED-00-40), March 14, 2000, at 6 (hereafter "GAO, *Safety of Farmworkers and Their Children"*).

[30] NRDC, *Farm Kids Petition*, at 2-3.

applying pesticides. Additionally, 40% of the children had entered fields that were still wet with pesticides and 40% had been sprayed with pesticides while in the fields.[31]

In the case of farmers and farm workers, a wide range of agricultural pesticides may be tracked into the home on shoes and brought into the home on clothing. A report of pilot portions of the Agricultural Health Study in Minnesota, Iowa, and North Carolina reported on analyses of carpet dust performed before, during, and after pesticide applications on the farm. House dust levels of herbicides including alachlor, metolachlor, atrazine, and 2,4-D increased by 10-100 fold in one home following field applications.[32]

On three farms, investigators detected a total of 17 different pesticides on the hands of children ranging from age three to age 15.[33] These exposures were not to workers but rather to non-working children. Nine pesticides, including alachlor, atrazine, 2,4-D, dicamba, pentachlorophenol, captan, chlorpyrifos, propoxur, and DDT were all found on the hands of a three-year-old child living on a farm.[34]

A recent Canadian study assessing the exposure of farm children to pesticides found detectable residues of 2,4-D in the urine of 30% of children, with maximum values as high as 100 µg/L, even though these children were not directly involved in handling the pesticide.[35]

Children have unique exposure patterns and sensitivities to pesticides. Per pound of body weight, children eat, drink, and breathe more than adults. Children also engage in more frequent hand-to-mouth contact, and therefore have higher rates of oral exposure from objects, dust, or soil.[36] The GAO found that crawling, sitting, and lying on contaminated surfaces may also increase exposure rates of farm children to pesticides.[37] Furthermore, as the GAO concluded, "[b]ecause young children's internal organs and

---

[31] Pollack, S., et al. Pesticide Exposure and Working Conditions among Migrant Farmworker Children in Western New York State. American Public Health Association Annual Meeting Abstracts 1990.
[32] Camann DE, Akland GG, Buckley JD, Bond AE, Mage DT. 1997. Carpet dust and pesticide exposure of farm children, Intl Soc Exp Anal Ann Mtg, Research Triangle Park, NC, November 5, 1997.
[33] Geno P, Camann D, Harding H, Villalobos K, Lewis R. 1996. Handwipe sampling and analysis procedure for the measurement of dermal contact with pesticides. Arch Environ Contam Toxicol 30:132-138.
[34] *Id.*
[35] Arbuckle TE, Cole DC, Ritter L, Ripley BD. 2004. Farm children's exposure to herbicides: comparison of biomonitoring and questionnaire data. Epidemiology 15(2):187-94.
[36] NRDC, *Farm Kids Petition*, at 3; GAO, *Safety of Farmworkers and Their Children*, at 17.
[37] GAO, *Safety of Farmworkers and Their Children*, at 17.

bodily processes are still developing and maturing, their enzymatic, metabolic, and immune systems may provide less natural protection than those of an adult.".

EPA's regulation establishing tolerances for 2,4-D fails to consider information concerning the sensitivities and exposures of farm children as a major identifiable subgroup. 67 Fed. Reg. 10622. Under 21 U.S.C. § 346a(b)(2)(D), EPA must consider data regarding farm children's dietary consumption patterns, aggregate exposure levels, and sensitivities to exposure. If reliable data are lacking, EPA should require the pesticide chemical registrant to secure the necessary data and should not reregister 2,4-D or issue new tolerances until such data are available.

(3) Farmworker/Occupational Exposure Underestimated

The assumption of an eight-hour work day is unrealistic and underestimates exposure

The EPA underestimates workers' exposure to 2,4-D–and hence improperly underestimates the risks of using the pesticide–by relying on faulty assumptions. The EPA's risk assessment for handlers is predicated on the assumption that workers are exposed to 2,4-D for only 8 hours per day. This assumption is inconsistent with farm labor reality. According to the National Agricultural Worker Survey (NAWS), conducted by the U.S. Department of Labor, the majority of farmworkers (56%) worked on average between 30 and 50 hours per week (in 1997-98); and 15% worked an average of more than 50 hours per week.[38]

Furthermore, exposure to 2,4-D continues after the work hours until farmworkers bathe and change out of contaminated clothing. Since the vast majority of farms do not provide showers for their handlers at the job site, exposure for all handlers continues until they return to their living quarters, bathe and change clothes. One study of settled farmworkers in the Yakima Valley found that only 50% of agricultural workers bathed and changed clothes as soon as they returned home. When only half the settled workers bathe immediately, that indicates that among migrant workers who may live in shacks, their cars or even the fields–and often lack ready access to shower facilities–the number who wear their clothes all evening before changing would be far higher. In addition,

[38] U.S. Department of Labor, Office of Assistant Secretary for Policy, Findings from the National Agricultural Workers Survey 1997-98, Research Paper No. 8 March 2000 at p. 32.

some farmworkers sleep in their work clothes and/or wear the same contaminated clothing all week long. The unfortunate reality is that farmworkers are often too poor to have multiple work outfits, and their living conditions do not readily allow them to wash their clothes during the workweek. Additionally, when evaluating the true health risks to handlers, the EPA should consider that in many instances personal protective equipment (PPE) or engineering controls are not provided or may not work properly (e.g on hot days a handler may open the windows of a closed cab). Thus, in real terms, the risk to handlers will often exceed the risks demonstrated by the Margin of Exposure (MOE) calculations.

Occupational MOEs overestimate safety

The MOEs occupational handlers were calculated using a dermal absorption rate of 5.8% which is likely to lead to a serious underestimate of real exposure scenarios if dermal exposure is enhanced by the synergistic effects of sunscreens or insect repellents or from occlusion by clothing (see Section 4 on dermal absorption). Therefore, all MOEs for occupational exposure should be recalculated using modified values for dermal absorption.

Postapplication exposure and risk to workers underestimated

EPA's assumption that postapplication inhalation exposures are unlikely for workers is seriously flawed (particularly in the absence of any data addressing that specific exposure). The assumption appears to be based entirely on the "low vapor pressure of 2,4-D" (p. 84) but fails to take into account factors discussed in previous sections: Extended exposure to contaminated clothing and inhalation exposure to spray drift, volatized 2,4-D, and contaminated dust. Applications to any field or site with exposed soil (especially under dry or windy weather conditions) will almost certainly result in postapplication aerial movement (and inhalation) of contaminated soil.

Given the still unaddressed or incompletely quantified acute risks and ample data suggesting that 2,4-D is linked to a number of chronic diseases including cancer, the reentry interval (REI) for field workers should be at least 7 days. This would be an increase from the current REI of 12 hours for the ester and sodium salt forms of 2,4-D

and 48 hours for the acid and amine salt forms. Upon reclassification, other appropriate protections for workers will also be expected.

Unacceptable risks to workers warrants disallowance of some formulations

The MOE for both short- and intermediate-term exposures of mixers/loaders using wettable powder formulations for *all* crops both with and without PPE were far less than 100 which is the minimum level required by the Agency for safety. Similarly, the MOEs for application of liquid formulations applied to all crops and submerged aquatic weeds using baseline PPE were also far below the "safety" level of 100.

Therefore, there is no acceptable application for wettable powders with the exception of wettable powders packaged in water-soluble bags. In addition, all liquid formulations must be restricted to applications only when use of adequate PPE can be assured-i.e. when weather conditions and/or lack of access to adequate PPE are not realistic barriers.

(4) Dermal absorption of 2,4-D is underestimated

EPA used an outdated study to estimate dermal absorption of 2,4-D. The 1974 Maibach and Feldman study estimated a 5.8% dermal absorption through non-occluded intact skin. In fact, more recent studies have shown that such a low dermal absorption rate is unrealistically low and therefore insufficiently protective, especially due to the synergistic effects of other exposures. For example, the presence of DEET or sunscreen on the skin has been shown to significantly enhance absorption of 2,4-D. One study demonstrated 14% palmar absorption of 2,4-D after skin application of DEET.[39] Studies have also shown that most commercial sunscreen formulations enhance the penetration of 2,4-D through hairless mouse skin. One such study found that sunscreens increase penetration of 2,4-D by over 60 percent, from an average penetration of 54.9% to 86.9%.[40] Another study found more than a doubling in absorption from an average penetration of 39.1% for the no sunscreen control to 81.0% for mice pre-treated with

[39] Moody RP, Wester RC, Melendres JL, Maibach HI. 1992. Dermal absorption of the phenoxy herbicide 2,4-D dimethylamine in humans: effect of DEET and anatomic site. J Toxicol Environ Health 36(3):241-50.

[40] Pont AR, Charron AR, Brand RM. 2004. Active ingredients in sunscreens act as topical penetration enhancers for the herbicide 2,4-dichlorophenoxyacetic acid. Toxicol Appl Pharmacol 195:348-54.

Neutrogena Oil Free Sunscreen.[41] These results in the mouse appear also to be relevant to humans.[40] In addition to penetration enhancement due to commonly-applied topical products, one study in rodents has demonstrated a 2.2-fold enhancement in dermal absorption after regular ethanol consumption over a 6 to 8 week period.[42]

The study used by EPA to estimate dermal absorption also did not use any form of occlusion over the applied 2,4-D. Therefore the effect of 2,4-D that soaks clothing, or is subsequently covered by clothing or gloves would not be adequately assessed. Existing research on other chemicals indicates that occlusion is known to significantly enhance skin absorption of dermally-applied materials.[43]

Toddlers are a population of special concern for dermal exposure and have not been studied for dermal penetration of 2,4-D. The skin surface area of an infant per unit of body weight is double that of an adult.[44] All studies which have investigated dermal exposures to pesticides in children have found that this is a major route of exposure. Hands moist with saliva collect about 100 times more pesticide residue than dry hands, and children's hands are much more likely to be moist.[45] As a result of these data, EPA should revise upward the dermal absorption factor used in the 2,4-D risk assessment, and should select a dermal absorption factor of at least 14% based on the data presented in Moody et al. (1992). Such an adjustment of the dermal absorption factor would significantly affect the MOEs for some of the endpoints. For example, the short-term and one-day toddler postapplication exposures to treated turf already are at the Agency's level of concern, and would exceed the level of concern if the effects of DEET, sunscreen, or moist skin were included in the dermal absorption factor.

---

[41] Brand RM, Spalding M, Mueller C. 2002. Sunscreens can increase dermal penetration of 2,4-dichlorophenoxyacetic acid. J Toxicol Clin Toxicol 40(7):827-32.
[42] Brand RM, Charron AR, Dutton L, Gavlik TL, et al. 2004. Effects of chronic alcohol consumption on dermal penetration of pesticides in rats. J Toxicol Environ Health A 67(2):153-61.
[43] Riviere JE, Baynes RE, Brooks JD, Yeatts JL, Monteiro-Riviere NA. Percutaneous absorption of topical N,N-diethyl-m-toluamide (DEET): effects of exposure variables and coadministered toxicants. 2003. J Toxicol Environ Health A. 66(2):133-51.
[44] NRC. 1993. Pesticides in the Diets of Infants and Children. Washington DC: National Academy Press.
[45] Camann DE, Majumdar TK, Harding HJ, Ellenson WD, Lewis RG. 1996. Transfer efficiency of pesticides from carpet to saliva-moistened hands. Measurements of Toxic and Related Air Pollutants; VIP-64:532-540.

(5) Acute risk to toddlers playing on lawns is underestimated

In the risk assessment, section 4.4.2.2., EPA calculates exposure and risk estimates for residential turf application scenarios involving toddlers. In a bizarre twist, the Agency switches NOAELs during the analysis in a manner that barely manages to maintain the total calculated MOE at the Agency's level of concern. Rather than using the short-term NOAEL of 25 mg/kg/day based on the rat developmental toxicity study, and endpoint used for the other calculations, the EPA selects the NOAEL of 67 mg/kg/day based on the adult acute neurotoxicity study. The switch from one endpoint to the other is not justifiable, although the Agency argues (in the complete absence of supporting data) that the developmental toxicity endpoint "would only occur after several days of exposure". If EPA is to adopt this position, it must justify and explain why it is using an adult neurotoxicity test to assess risks to the toddler, when the Agency has already acknowledged the need for a developmental neurotoxicity test, and admitted that the potential neurotoxicity to infants and children has not been adequately assessed. Such a choice of NOAEL is reckless, and certainly not health-protective to this most vulnerable of subgroups. According to our review of this analysis, it seems clear that lawn application of 2,4-D is dangerous to toddlers and should not be legally allowed.

(6) Exposure and risks underestimated for recreational swimmers

In order to determine postapplication-aquatic use risks, the agency uses the maximum water concentrations for adults, but only the "target concentration" for children. Yet, the agency also acknowledges that, "2,4-D concentrations sometimes exceeded the target concentration in parts of the treated area shortly after application." (p.12). It is unclear why maximum water concentrations are used for adults and target concentrations for children. It is extremely important that children receive maximum protection in post-treated waters and that their risk is determined based on maximum exposure, particularly because the Agency has found children to be much more vulnerable. Therefore, it appears that maximum water concentrations should be used for children as well as adults. We are concerned that as a result of this risk assessment, mitigation measures may be adopted that are not protective enough. If the exposure level

in the risk assessment is not a legally enforceable level, then by virtue of the risk assessment calculation, people can be harmed.

(7) Deficient reasoning and data gap in dissipation rates underestimates risks to swimmers in treated waters

The agency makes the following statements regarding exposure through swimming/water: "MOEs for toddler short-term exposures were also calculated using the target concentration (because there was insufficient data to define a dissipation rate) along with the short term NOAEL." (p.12) "The probability that a person would swim in an area recently treated for milfoil is low because the presence of milfoil makes swimming difficult and unpleasant." (p.13).

While it is unlikely that people swim in the milfoil, it is highly likely that they could swim near it in a lake. It is unclear from the risk assessment documents how far the herbicide travels from the target site once in the aquatic environment to the point of dissipation. It is not clear what field dissipation studies were used by the agency and why "there was insufficient data to define a dissipation rate" (p.12). Without adequate dissipation rates, it is impossible to assess the exposure levels for adults or children either near the plant or any given distance from it, especially considering that the half-lives are lengthy (2.9 to 29.5 days with an average of 11.4 days and a geometric mean of 7.3 days). The assumption that people, particularly children, will not swim near (as opposed to in) treated milfoil is not protective and is an insufficient reason to disregard the need for accurate dissipation rates. We identify dissipation rates as a serious data gap that should be addressed prior to any action by the agency. The absence of such data coupled with the agency's deficient reasoning of unlikely exposure in water where milfoil is treated will essentially lead to policy decisions that lack adequate warnings or other mitigation measures.

(8) Agency must *require* additional studies on inhalation risks

We strongly support the requirement of a subchronic inhalation study for 2,4-D to fill the data gap on repeated inhalation exposure. The Agency identifies the serious data gap and quantifies it by stating that, "the only reliable way to characterize inhalation

toxicity and to quantify inhalation risk is through the use of inhalation toxicity studies." The Agency further supports the need for additional research by stating that, "chemicals tend to be more toxic by the inhalation route than by the oral route due to rapid absorption and distribution, bypassing of the liver's metabolic protection (portal circulation), and potentially serious portal-of-entry effects, such as irritation, edema, cellular transformation, degeneration, and necrosis. An inhalation risk assessment that is based on oral data generally underestimates the inhalation risk because it cannot account for these factors" (p. 6).

Atmospheric transport of 2,4-D includes spray drift at the time of application, volatilization and transport of 2,4-D on dust particles (EFED, p.40).[46,47] While the Agency considers that spray drift has been well-studied (and included in EFED's risk assessment models), "transport after volatilization is not as well studied and the impact of the potential transport of 2,4-D esters away from the target site is not included quantitatively in [the EFED] assessment" (EFED, p.40). There is no mention of other forms of 2,4-D (acids, salts and other esters).

From 1996 to 1998, 2,4-D was the most commonly confirmed active ingredient by state agencies in regards to drift complaints.[48] A study of transport of 2,4-D into homes (in air or on dust particles) for up to one week following lawn applications indicated that post application exposure levels to young children were about 10 times the pre-application levels and at least an order of magnitude greater than dietary exposures. Resuspension of floor dust was the major source of 2,4-D in indoor air.[49] See Section 11 on Aggregate Risk for more detail.

In the Agency's assessment of the one-day/short-term residential postapplication exposure, MOEs for toddlers were 1,000 just matching the Agency's level of concern (p.66-67). Had the appropriate assumptions regarding inhalation from tracked-in 2,4-D

[46] Environmental Fate and Effects Division's Risk Assessment for the Reregistration Eligibility Document for 2,4-Dichlorophenoxyacetic Acid (2,4-D)
[47] Nishioka MG, Lewis RG, Brinkman MC, Burkholder HM, Hines CE, Menkedick JR. 2001. Distribution of 2,4-D in air and on surfaces inside residences after lawn applications: Comparing exposure estimates from various media for young children. Environ Health Perspect 109:1185-1191.
[48] Association of American Pesticide Control Officials (AAPCO) 1999 Pesticide Drift Enforcement Survey, available online at: http://aapco.ceris.purdue.edu/htm/survey.htm.
[49] Nishioka et al., 2001.

and the modified dermal absorption used (considering effects of sunscreen, DEET and/or occluding clothes) then the MOEs would have exceeded the level of concern.

It is unclear whether the Agency is requiring or recommending a subchronic inhalation study. On page 6 EPA *requires* a subchronic study to be done but on page 89 the Agency *recommends* the study. Given the undisputed data gaps on subchronic *and* short-term inhalation exposure for 2,4-D we support the Agency's strict requirement for this study and find it unacceptable that EPA would propose the possibility of a rationale that would allow the study to be waived. ..

(9) Toxicity of 2,4-D in dogs was incorrectly disregarded

EPA chooses to assess human health risk on the basis of rodent studies and disregards the toxicity of this chemical in dogs, even though basing the risk assessment on the dog would clearly be more health-protective. The Agency presents three main arguments from the pesticide registrants to support disregarding the dog data. The arguments include: (1) the dog has decreased clearance of 2,4-D compared to rats, mice, and humans; (2) the half-life of elimination of 2,4-D is longer in the dog than in mice, rats, pigs, cats, and humans; and (3) the difference in the elimination pattern among dogs, rats, and other species persuaded the Agency that the rat is a better predictor than the dog of potential human toxicity. It is apparent that all three of the above arguments are essentially three statements of the same argument, and that all of the arguments are based completely on an argument about excretion of 2,4-D and the resulting half-life of the chemical in the body.

We acknowledge that there are data showing that dogs appear to excrete 2,4-D slowly, and that the longer plasma half-life of 2,4-D in dogs is likely to partly or largely explain the increased toxicity observed in this species. However, we do not believe that the data support the registrant's (and EPA's) contention that the human resembles the rat. Table 1 below summarizes the plasma half-life of 2,4-D in the rat, the human, and the dog.[50] Although the half-life is between 10 and 100-fold longer in the dog than in the rat, it is clear that in the human, the plasma half-life of 2,4-D is intermediate between the two

[50] Timchalk C. 2004. Comparative inter-species pharmacokinetics of phenoxyacetic acid herbicides and related organic acids. Evidence that the dog is not a relevant species for evaluation of human health risk. Toxicology 200(1):1-19.

other species. For example, although the half-life of 2,4-D is 8-fold longer in the dog than in the human, it is also 12-fold longer in the human than in the rat. Therefore the human would be expected to be intermediate in susceptibility between the rat and the dog. There are several ways that EPA could account for this problem, but simply assuming that the human is like the rat is not a scientifically-defensible option.

We advocate using the dog data because doing so is more precautionary and would help protect vulnerable members of the human population from 2,4-D. Alternatively, EPA could select as a NOAEL a number intermediate between the NOAEL found in the dog studies and that found in the rat studies. Such an intermediate NOAEL could be justified based on the evidence that the human is likely to be intermediate in susceptibility between the rat and the dog.

Table 1:

Species	2,4-D half-life in plasma (hours)	MCPA half-life in plasma (hours)
Rat	1	6
Human	12	11
Dog	96	63

Source: Timchalk C. Toxicology 200:1-19, 2004

(10) Evidence that 2,4-D is mutagenic and genotoxic is disregarded

The discussion on the carcinogenicity/mutagenicity of 2,4-D on page 33 of the EPA risk assessment is inadequate. Although EPA does acknowledge some positive mutagenicity and cytogenicity studies (e.g. in *Drosophila* larvae, in mammalian cell cytogenics assays after metabolic activation) the Agency fails to acknowledge numerous additional positive studies in the peer-reviewed scientific literature that together indicate that 2,4-D is likely to be cytotoxic and mutagenic. For example, Zeljezic and colleague (2004) tested a commercial formulation of 2,4-D on human lymphocytes and found a treatment-related elevation in the number of chromatid and chromosome breaks, as well as acentric fragments and aberrant cells at concentrations of 0.4 µg/ml.[51] Metabolic activation significantly increased the frequency of chromatid and chromosome breaks.

[51] Zeljezic D, Garaj-Vrhovac V. 2004. Chromosomal aberrations, micronuclei and nuclear buds induced in human lymphocytes by 2,4-dichlorophenoxyacetic acid pesticide formulation. Toxicology 200:39-47.

The same researchers reported significant increases in the number of micronuclei and nuclear buds at this dose level. A relatively recent study by Arias (2003) found a significantly higher rate of sister chromatid exchange (SCE) in chick embryos treated with 2,4-D and its isooctyl ester.[52] Another study, by Madrigal-Bujaidar and colleagues (2001), also reported an increased frequency of SCE in bone marrow and spermatogonial cells of mice exposed in vivo to 100 mg/kg of 2,4-D.[53] Other researchers have tested 2,4-D in yeast, transformed hematopoietic cells, and mouse bone marrow, and have found both cytotoxic and mutagenic effects, including chromosomal breaks, deletions, and exchanges.[54] Tests in Drosophila have also demonstrated genotoxicity to both somatic and germ-line cells.[55]

Other researchers publishing in the open scientific literature have reported oxidant effects of 2,4-D, indicating the potential for cytotoxicity or genotoxicity. For example, Bukowska (2003) reported that treatment of human erythrocytes in vitro with 2,4-D at 250 and 500 ppm resulted in decreased levels of reduced glutathione, decreased activity of superoxide dismutase, and increased levels of glutathione peroxidase.[56] These significant changes in antioxidant enzyme activities and evidence of oxidative stress indicate that 2,4-D should be taken seriously as a cytotoxic and potentially genotoxic agent. The cytotoxicity of 2,4-D was demonstrated in human hepatoma cells where treatment resulted in significantly increased rates of apoptosis related to a breakdown of mitochondrial membrane potential, the induction of DNA strand breaks, and a loss of membrane integrity.[57] The authors of this study concluded that 2,4-D is a cytotoxic agent.

---

[52] Arias E. 2003. Sister chromatid exchange induction by the herbicide 2,4-dichlorophenoxyacetic acid in chick embryos. Ecotoxicol Environ Saf 55(3):338-43.
[53] Madrigal-Bujaidar E, Hernandez-Ceruelos A, Chamorro G. 2001. Induction of sister chromatid exchanges by 2,4-dichlorophenoxyacetic acid in somatic and germ cells of mice exposed in vivo. Food Chem Toxicol 39(9): 941-6.
[54] Venkov P, Topashka-Ancheva M, Georgieva M, Alexieva V, Karanov E. 2000. Genotoxic effect of substituted phenoxyacetic acids. Arch Toxicol 74:560-6.
[55] Tripathy NK, Routray PK, Sahu GP, Kumar AA. 1993. Genotoxicity of 2,4-dichlorophenoxyacetic acid tested in somatic and germ-line cells of Drosophila. Mutat Res 319(3):237-42.
[56] Bukowska B. 2003. Effects of 2,4-D and its metabolite 2,4-dichlorophenol on antioxidant enzymes and level of glutathione in human erythrocytes. Comp Biochem Physiol C Toxicol Pharmacol 135(4):435-41.
[57] Tuschl H, Schwab C. 2003. Cytotoxic effects of the herbicide 2,4-dichlorophenoxyacetic acid in HepG2 cells. Food Chem Toxicol 41:385-393.

Further, De Moliner, et al. (2002) found apoptosis or genetically determined programmed cell death in brain cells, further confirming the cytotoxicity of 2,4-D.[58]

Some researchers have hypothesized that some of the apparently discrepant results on mutagenicity and genotoxicity may be related to the formulations tested, since 'other' ingredients in the formulation may enhance the mutagenicity or genotoxicity of the active ingredient.[59] From a practical and legal viewpoint, however, it is the cumulative risk to the end user that is at issue here and therefore EPA should take this concern seriously and should, if necessary, require additional testing to resolve the conflicts in the database.

Another finding that may provide a unifying explanation of some of the data on 2,4-D and lymphoma, is that the herbicide may increase lymphocyte replication. One longitudinal study of pesticide applicators found urine concentrations of 2,4-D ranging from 1.0 to 1700 µg/g creatinine/L urine that logarithmically increased as spraying time increased. In addition to suggesting increasing risk of chronic toxicity to pesticide applicators due to the apparent exceedence of human renal clearance mechanisms, this study found increasing lymphocyte replicative index (of 11-14%) in these applicators in a manner that was directly related to 2,4-D absorbed dose.[60] This finding was confirmed *in vivo* and *in vitro* in a follow-up study, showing a 12-15% increase in replicative index at an 0.005 mM exposure to 2,4-D, with an indication that higher-dose exposures may exhibit a direct cytotoxic effect on lymphocytes that results in a decreased replicative index, resulting in an inverted U-shaped dose-response curve.[59] The consistency of these findings indicate that 2,4-D may have an immunotoxic effect that alters replication of human lymphocytes, thereby increasing the risk of lymphoid cancer in humans. This finding would be consistent with the frequently-reported epidemiologic evidence linking 2,4-D exposure to NHL in humans. Unfortunately, EPA failed to mention any of this information in its risk assessment of 2,4-D, thereby failing to fully assess the risk of

[58] De Moliner KL, de Duffard AME, Soto E, Duffard R, Adamo AM. 2002. Induction of apoptosis in cerebellar granule cells by 2,4-dichlorophenoxyacetic acid. Neurochem Res 27:1439-1446

[59] Holland NT, Duramad P, Rothman N, Figgs LW, et al. 2002. Micronucleus frequency and proliferation in human lymphocytes after exposure to herbicide 2,4-dichlorophenoxyacetic acid in vitro and in vivo. Mutation Research 521:165-178.

[60] Figgs LW, Holland NT, Rothmann N, Zahm SH, et al. 2000. Increased lymphocyte replicative index following 2,4-dichlorophenoxyacetic acid herbicide exposure. Cancer Causes Control. 11(4):373-80.

cancer in humans from this exposure and failing to adequately protect humans from this risk.

Carcinogenicity classification

The 2,4-D 'Toxicology Disciplinary' chapter provides the literature review that the Agency considered for 2,4-D toxicology and risks. In contrast to the hundreds of high-quality published studies (see Appendix A), the Agency considered a mere 11 studies from the published literature. In light of the unique body of literature and epidemiological data, produced by the National Cancer Institute among others, identifying cancer endpoints, particularly Non-Hodgkin's Lymphoma, a Class D is clearly inappropriate. The great numbers of positive evaluations of 2,4-D's carcinogenicity alone undermine the Agency's Class D categorization, which is designed for less-studied chemicals. Evaluation of this evidence compels a carcinogenicity classification of at least a Class C.

We request the Agency to please fully justify a D classification, including why epidemiological studies are disregarded and why the Agency does not appear to consider the quality the evidence (as measured by the proxy of the quality of peer review). We also request the Agency to please explain its claim that "The endpoint selected for the cPAD will be protective of the possible carcinogenic activity of this chemical" (p.79) when according to the Agency, there is insufficient evidence to classify 2,4-D as a carcinogen.

We view the risk assessment and reregistration process of 2,4-D as an important opportunity for the Agency to utilize the abundance of relevant data to fulfill its mandate in providing serious protection to human health and the environment from cancer and other illnesses.

Regarding the di-ethanol-amine (DEA) form of 2,4-D, we would appreciate the opportunity to review and comment on the submissions of data from industry through a public notice and comment period before the completion of the Agency's overall evaluation of this chemical.

Failure to assess dioxin contaminants

It is unacceptable that the Agency failed to examine or acknowledge the possibility of dioxin contamination in 2,4-D formulations in its Health Effects Division or Toxicology Disciplinary chapters. Dioxin was mentioned in the Environmental Fate and Effects Division (EFED) Revised chapter in just two sentences. It reads, "Key findings of this risk assessment are as follows: Dioxin congeners have been identified as a by-product in the production of 2,4-D and its various chemical forms and has been detected in the technical formulations. The potential ecological impact of dioxin in 2,4-D will be addressed in a separate document" (EFED, p.6). Would the Agency please explain the timetable on this document and who will be in charge of its completion? Would the Agency also please explain why dioxin contamination of 2,4-D products was not fully addressed in this risk assessment?

The Agency has a statutory responsibility under Section 408(b)(2)(D)(v) of the FFDCA (see Section 12 below for more discussion) to examine and assess the effects from the range of dioxin contaminants in various 2,4-D products as well as the interaction of the active, inert, and contaminant ingredients in 2,4-D with its commonly associated phenoxy herbicides such as dicamba and mecoprop.[61] In this process, it is essential to consider the risks from the relatively well-studied 2,3,7,8-Tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD), which is still found in some 2,4-D formulations and products, as well as to assess 1,2,3,7,8-Pentachlorodibenzo-p-dioxin. Both the former and latter dioxin or furan contaminants have been given the Toxic Equivalency Factor of "1" in the 2000 Toxics Inventory Release report.[62]

An acknowledgment of a dioxin finding in 2,4-D should be red flag to the HED. As the Agency is well aware, a complete risk assessment on 2,4-D for human health cannot be complete without a full analysis of the various dioxin contaminants in all formulations and batches of 2,4-D.[63] This analysis must include a thorough review of

[61] A compilation of documents by Health Canada's Pest Management Regulatory Agency has revealed that federal regulators have discovered traces of dioxins and furans in 10 pesticides currently used in Canada, including 2,4-D, dicamba and mecoprop.

[62] See Chapter 3: "PBT Chemicals: Dioxin and Dioxin-like Compounds."

[63] Some references relating to 2,4-D and/or 2,3,7,8-TCDD include: Cochrane, W., et al. 1981. "Determination of chlorinated dibenzo-p-dioxin contaminants in 2,4-D products by gas chromatography-mass spectrometric techniques." Journal of Chromatography 217:289-299. Hagenmeier, H. 1986. "Determination of 2,3,7,8-tetrachlorodibenzo-p-dioxin in commercial chlorophenols and related products."

studies relating to 2,4-D and its accompanied herbicides and contaminants, particularly concerning bioaccumulation and prolonged human exposure to 2,3,7,8-TCDD and other dioxins/furans, for reasons explained in Section 12 of this document.

The little testing that has been done on dioxin contamination shows that current 2,4-D products are contaminated with dioxins, including 2,3,7,8-TCDD, the most toxic dioxin. 2,3,7,8-TCDD was found in 2 of the 8 samples analyzed for EPA by 2,4-D manufacturers. A closely related dioxin (1,2,3,7,8-pentachlorodibenzo-p-dioxin) was found in 3 of the 8 samples tested.[64] Analysis provided by the Washington Department of Agriculture recently surveyed fertilizer products including one 2,4-D-containing product that showed 2,4-D contamination with 2,3,7,8-TCDD, the same pentadioxin found by EPA to be the other most toxic, as well as three related dioxins.[65]

Clearly any carcinogenic analysis of 2,4-D must consider dioxin/furan contamination. Can the Agency please explain its position in not assessing this most important and well-studied cancer risk?

(11) The aggregate risk assessment is inadequate

The FQPA, 21 U.S.C. § 346a(b)(2)(A)(ii) requires that, to establish a pesticide tolerance, there must be a "reasonable certainty that no harm will result from aggregate exposure to pesticide chemical residue, including all anticipated dietary exposures and other exposures for which there are reliable information." Aggregate exposure is the total exposure to a single chemical or its residues that may occur from dietary (*i.e.*, food and drinking water), residential, and all known or plausible exposure routes (including oral, dermal and inhalation). *See id.* Therefore, in addition to food and water exposures, the aggregate assessment must take into account exposures due to air drift and migration of contaminated soil, residential exposures from registered uses, and residential "take-home" exposures to families of those directly exposed to the pesticides through its

---

Journal of American Medical Association 171(10): 1306-1308. U.S. EPA. 1981. Memorandum Analysis for di- and tetra-chlorinated dibenzo-p-dioxins in 2,4-D, from R. Harless to Mike Dellarco. Office of Toxic Substances. Washington, D.C. As cited in Banes, 1991.

[64] U.S. EPA. Office of Research and Development. 1994. Estimating exposure to dioxin-like compounds. Vol. II: Properties, sources, occurrence and background exposures. Review draft. Washington, D.C., June.

[65] Washington Depts. of Ecology, Agriculture, and Health. 1998. Screening survey for metals and dioxins in fertilizers, soil amendments, and soils in Washington State. Olympia WA, Nov.

agricultural uses. Furthermore, the aggregate assessment must consider exposures from uses that do not conform with the label, if there is an indication that such uses occur.

EPA failed to conduct an adequate aggregate assessment in this risk assessment for 2,4-D. EPA's risk assessment does not consider exposure through air drift, migration of contaminated soil, or residential track-in exposures. In addition, there are reliable data concerning take-home exposure to pesticides resulting from agricultural uses.[66]

Ample data demonstrate that 2,4-D migrates indoors after application on lawns. One investigation revealed that 3% of dislodgeable residues of 2,4-D on a lawn was tracked indoors and accumulated in carpet dust.[67] Although 2,4-D normally degrades relatively rapidly outside, it has been shown to linger in the indoor environment.[68] Calculations based on a single lawn application of 2,4-D indicate that detectable levels of the pesticide would remain in carpet dust up to one year after a one-time lawn application.[69] An in-depth study of a home in San Antonio, Texas, revealed detectable residues of 16 pesticides in the living room carpet. Gradients of many of these pesticides were apparent from the lawn and garden onto the front doorstep and into the carpet indicating that the pesticides were transported into the home primarily on shoes.[70] A study published recently in Environmental Health Perspectives, found that track-in by an active dog and by the homeowner applicator were the most significant factors for intrusion of 2,4-D into the home. Resuspension of floor dust was the major source of 2,4-D in indoor air, with highest levels of 2,4-D found in the particle size range of 2.5-10

---

[66] Lu C, Knutson DE, Fisker-Andersen J, Fenske RA. 2001. Biological monitoring survey of organophosphorus pesticide exposure among pre-school children in the Seattle metropolitan area. Environ Health Perspect 109:299-303.

[67] Nishioka M, Burkholder H, Brinkman M, Gordon S. 1996. Measuring Transport of Lawn-Applied Herbicide Acids from Turf to Home: Correlation of Dislodgeable 2,4-D Turf Residues with Carpet Dust and Carpet Surface Residues. Environ Sci Technol 30:3313-3320; Nishioka MG, Lewis RG, Brinkman MC, Burkholder HM, Hines CE, Menkedick JR. 2001. Distribution of 2,4-D in air and on surfaces inside residences after lawn applications: comparing exposure estimates from various media for young children. Environ Health Perspect 109(11):1185-91.

[68] Lewis R, Bond A, Fortmann R, Sheldon L, Camann D. 1991. Determination of routes of exposure of infants and toddlers to household pesticides: a pilot study to test methods, Air and Waste Management Association, 84th Annual Meeting, Vancouver, British Columbia.

[69] Nishioka M, Burkholder H, Brinkman M, Gordon S. 1996. Measuring Transport of Lawn-Applied Herbicide Acids from Turf to Home: Correlation of Dislodgeable 2,4-D Turf Residues with Carpet Dust and Carpet Surface Residues. Environ Sci Technol 30:3313-3320.

[70] Camann D, Lewis R. 1990. Trapping of particle-associated pesticides in indoor air by polyurethane foam and exploration of soil track-in as a pesticide source, Indoor Air '90: Proc 5th Intl Conf on Indoor Air Quality and Climate, Toronto, Vol. 2.

microns. Resuspended floor dust was also a major source of 2,4-D on tables and window sills. Estimated postapplication indoor exposure levels for young children from nondietary ingestion were 1-10 μg/day from contact with floors, and 0.2-30 μg/day from contact with table tops. These are estimated to be about 10 times higher than the preapplication exposures. By comparison, dietary ingestion of 2,4-D is approximately 1.3 μg/day.[71] Thus, "tracking-in" of pesticides is likely to be both common and significant, and must be included in the aggregate risk assessment.

The above deficiencies reveal that EPA improperly underestimated aggregate exposure to 2,4-D and its residues that may occur from dietary, residential, and all other known or plausible exposure routes. The use of 2,4-D in and around the home could itself exceed appropriate risk levels if properly calculated. The assumptions and missing data in EPA's analysis of aggregate exposure for 2,4-D serve to underestimate exposure and therefore underestimate risk, contrary to the requirements of the FQPA.

(12) Agency has a statutory obligation to assess combined effects with other chemicals

The passage of the *Food Quality Protection Act of 1996* (FQPA) ushered in a new set of responsibilities for the EPA under the *Federal Food, Drug, and Cosmetic Act* (FFDCA). One of the most important of those new responsibilities is the requirement that EPA assess the risks associated with exposure to multiple chemicals from multiple pathways. In the HED chapter, the Agency has recognizes its legal responsibility under Section 408(b)(2)(D)(v) of the FFDCA which requires that, when considering whether to establish, modify, or revoke a tolerance, the Agency consider "available information" concerning the cumulative effects of a particular pesticide's residues and "other substances that have a common mechanism of toxicity." (p.79)

It is also important to note the section just prior to the one above concerning proper assessment of cumulative effects on infants and children: The agency shall assess the risk of the pesticide chemical residue based on "available information concerning the

[71] Nishioka MG, Lewis RG, Brinkman MC, Burkholder HM, Hines CE, Menkedick JR. 2001. Distribution of 2,4-D in air and on surfaces inside residences after lawn applications: comparing exposure estimates from various media for young children. Environ Health Perspect 109(11):1185-91.

cumulative effects on infants and children of such residues and other substances that have a common mechanism of toxicity." Section 408(b)(2)(C)(i)(III).

Furthermore, under the *Federal Insecticide, Fungicide, And Rodenticide Act* (FIFRA), as amended by the FQPA, Section 3(c)(5)(D) the law requires that upon registering a pesticide, the Agency must assess that: "when used in accordance with widespread and commonly recognized practice it will not generally cause unreasonable adverse effects on the environment."

Yet, the Agency attempts to justify not performing a cumulative risk assessment as part of this 2,4-D human health risk assessment "because the Agency has not yet made a determination as to which compounds to which humans may be exposed, if any, have a common mechanism of toxicity." (p.79)

The Agency is clearly out of compliance considering this chemical's relatively unique characteristic of both common use and common mechanism of toxicity of the chlorophenoxy herbicides found in most end use 2,4-D products. We believe there is sufficient evidence that several of the chlorophenoxy herbicides used in combination with 2,4-D have common mechanisms of toxicity and therefore, need to be assessed in light of the combined toxicities. And even if the Agency continues to dispute the common mechanism of toxicity, it is still obligated to consider the combined toxicities that result from the way 2,4-D is commonly used (and the public and the environment is commonly exposed).

In assessing the residential applicator exposures and risks, HED section 4.4.1 states, "According to the EPA Pesticide Sales and Usage Report for 1998/1999, 2,4-D is the most commonly used conventional pesticide active ingredient in the home and garden market sector with 7 to 9 million pounds applied per year." "The residential products are typically formulated as dry weed and feed products or as liquids in concentrates or ready to use sprays. Many of these formulations include other phenoxy herbicides such as MCPP-p and dicamba." (p. 62)

In support of the agency's above observation, a recent shelf survey of retail stores in the San Francisco Bay Area, every product that contained 2,4-D also contained at least

one other chlorophenoxy herbicide, usually MCPP or MCPP-p.[72] Similarly, another survey of retail stores in the Seattle area identified a total of 28 products containing 2,4-D. Only two of those products contained 2,4-D alone. Twenty-five products contained MCPP in addition to 2,4-D, and 14 also contained dicamba. The average ratio between MCPP and 2,4-D in these products was 1.12:1; on average products contained roughly 12% more MCPP than 2,4-D.[73]

Trimec™ is a common trade name for certain combinations of 2,4-D with two other active ingredients. Trimec is sold alone as well as formulated into other end-use products. EPA's registration database shows 84 active product registrations for products containing Trimec™.[74] All 84 of these products contain two phenoxy herbicides plus dicamba. Sixty-seven products contain both 2,4-D and MPCC, and on average the concentration of MCPP in those products is 69% of the concentration of 2,4-D.

It is impossible to predict the interactive effects that could result from a mixture of active ingredients. However, when data are available revealing the interactivity of a suite of chemicals often found in the environment, EPA should give these data high priority when determining risk.

Although the agency acknowledges that combinations of 2,4-D, a chlorophenoxy herbicide, typically contain MCPP and dicamba (also chlorophenoxy herbicides), the agency fails to meet its statutory responsibility to take into account realistic toxicity levels of combined exposure or even acknowledge the common mechanisms of toxicity and thus, does not assess the real potency of such combinations and added risks associated with exposure. While nothing in our comments should preclude the agency from taking immediate action to remove the risks associated with the active ingredient 2,4-D, the agency must assume its statutory responsibility to consider the cumulative risks of 2,4-D and similar active ingredients. Without a cumulative assessment of the combined effects with other chemicals, the risk assessment of 2,4-D will fail to calculate the real risks to human health and the environment as experienced in the real world.

---

[72] TDC Environmental. 2004. San Francisco Bay Area Pesticide Retail Store Survey. TDC Environmental. Report for the City of San Jose. June 2004.; Moran, K. 2004. Personal communication.
[73] Dickey, P. 2002. Grow Smart, Grow Safe. A Consumer Guide to Lawn and Garden Products. Local Hazardous Waste Management Program in King County. Fourth Edition.
[74] USEPA. 2004. U.S. Environmental Protection Agency/Office of Pesticide Programs. Pesticide Products Database Query. http://www.cdpr.ca.gov/docs/epa/m2.htm. (Accessed August 11, 2004)

There is considerable scientific support for the idea that chemicals with common toxicological modes of action, often predicted by structural similarities, usually have toxicity that is concentration additive.[75] If pesticide products contain multiple active ingredients with similar properties, it can be expected that human and environmental exposures will be in proportion to the makeup of the products. Water quality data from urban areas support this conclusion. In a study comparing pesticides in ten small streams during high flow conditions in 1997, both 2,4-D and MCPP were detected in all streams.[76] A sample-by-sample comparison of all samples where 2,4-D was detected showed a strong linear correlation between measured MCPP and 2,4-D concentrations, with a slope 0.65 indicating that MCPP concentrations are tending towards 65% of 2,4-D concentrations. The mean ratio of MCPP to 2,4-D on a per-sample basis was 1.13. These results are in close agreement with the relative percentages of MCPP and 2,4-D found in actual products.

Just considering the two ingredients MCPP and 2,4-D strongly suggests that simultaneous exposures to these two compounds will be the rule rather than the exception, and that it is plausible to assume that MCPP exposures to humans and aquatic species in urban areas are probably in the range of 60% to 120% of 2,4-D exposures.[77] This represents a substantial additional risk of chlorophenoxy herbicides that must be taken into account in the risk assessment for 2,4-D. The similarity in chemical structures for the compounds listed below is sufficient to warrant regulatory treatment as a group.

Common name(s)	Technical name
2,4-D	2,4-dichlorophenoxy acetic acid
2,4-DP, dichlorprop	2-(2,4-dichlorophenoxy)propanoic acid
MCPP, mecoprop	2-(4-chloro-2-methylphenoxy)propanoic acid

[75] Van Leeuwen, C.J., H.J.M Verhaar, and J.L.M. Hermens. 1996. Quality criteria and risk assessment for mixtures of chemicals in the aquatic environment. Human. Ecol. Risk Assess., 6: 419-425; Vighi, M and D. Calamari. 1996. Quality objectives for aquatic life: The problem of mixtures of chemical substances. Human. Ecol. Risk Assess., 6: 412-418; Grimme, L.H., M. Faust, W. Boedeker, and R. Altenburger R. 1996 Aquatic toxicity of chemical substances in combination: Still a matter of controversy. Human Ecol. Risk Assess., 2: 426-433.

[76] Voss, F.D. and S.S. Embrey. 1998. Pesticides detected in urban streams during rainstorms in King and Snohomish Counties, Washington, 1988. U.S. Geological Survey Water Resources Investigations Report 00-4098. 22 pages.

[77] Seattle shelf survey showed ratio of 1.12:1; Trimec ratio is 0.69; water quality samples when graphed show 0.65 ratio but when taken as pairs and averaged come out to 1.13. So the range of all these numbers is roughly 60-120%.

MCPA	(4-chloro-2-methylphenoxy)acetic acid
Dicamba	3,6-dichloro-2-methoxy)benzoic acid

When the scientific community attempts to measure the toxicity of similar compounds, they look at the structures and try to make assessments. Given the similarity of the structures of these phenoxy compounds, we request that the EPA follow the same basic protocol as the scientific community – particularly because these compounds are usually found in the same product.

It appears that EPA has assumed a crude additive risk for the human health risk assessments by assuming maximum allowed 2,4-D application rates—combination products have lower maximum 2,4-D application rates. This approach is flawed because it does not take account of the actual toxicity of the other ingredients as mandated by the FQPA. For the aquatic risk assessments, it does not appear that any consideration of other active ingredients is taken into account. Since EPA claims that it cannot model urban runoff, urban risks must be based on observed surface water concentrations. These risks are underestimated if co-contaminants in the streams are not considered.

Given the prevalence of multiple active ingredients in products containing chlorophenoxy herbicides and the clear similarity in chemical structures, it is imperative that EPA evaluate the cumulative risks of these ingredients together. At a minimum, risk assessments should assume additive toxicity for the MCPP, MCPP-p and dicamba compounds unless data show otherwise.

Hence, we ask that the agency revisit the 2002 study that found that exposure of pregnant mice to a common combination of phenoxy herbicides including in drinking water resulted in reduced litter sizes.[78] In section 4.10 of the 2,4-D Toxicology disciplinary chapter, the agency disregarded this study stating, "that the effects reported in this study cannot be attributed to 2,4-D since a mixture of chemicals [2,4-D, mecoprop, dicamba] was tested….the HIARC concluded that the study is not relevant for risk assessment." (Phase 2 Toxicology Chapter Revision, p.44). Again, we find this conclusion to be flawed and contradictory to the agency's responsibility mandated in both the FIFRA and FQPA statutes. The mixtures used in this study represent exposure to

[78] MF Cavieres, J. Jaeger J and W. Porter published in 2002 (Environ Health Perspect. 2002 Nov;110(11):1081-5.

ingredients that likely have a common mechanism of toxicity and that are found in some of the most commonly used lawn care herbicides. Another study found 2,4-D and 2,4-D common combinations to be developmental toxicants. This study further substantiates the use of the 10X safety factor.[79]

CONCLUSION

In summary, we hope we have made it quite clear to the EPA that we have extensive and serious concerns about this 2,4-D risk assessment. We have identified some major statutory violations, inconsistencies, data gaps, deficient reasoning and underestimated risks by the Agency. Considering that the Agency has not taken all exposures and risks into account, particularly for infants and children, nor considered the plethora of independent peer-reviewed studies documenting serious potential risks from exposure 2,4-D, we feel it is completely inappropriate classify 2,4-D as a Class D, no evidence of carcinogenicity. We ask the EPA to please take these comments into full review and consideration and realign its assessments accordingly. We are confident that once the many deficiencies are corrected and full exposure and risks are accounted for, it will be clear that the risks to human health and the environment from 2,4-D and 2,4-D products are unacceptable and unsafe for use – at any level.

Sincerely,

Beyond Pesticides
Shawnee Hoover, Projects Director
701 E Street, SE
Washington, DC 20003

Pesticide Action Network North America
Margaret Reeves, Ph.D., Staff Scientist
49 Powell St., Suite 500
San Francisco, CA 94102

Natural Resources Defense Council
Gina M. Solomon, M.D., M.P.H. Senior Scientist Assistant Clinical Professor of Medicine, U.C.S.F
111 Sutter Street, 20th Floor
San Francisco, CA 94104

TEDX, Inc. (The Endocrine Disruption Exchange)
Theo Colborn, PhD, President
Senior Fellow, World Wildlife Fund
P.O. Box 1407
Paonia, CO 81428

[79] 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.

Northwest Coalition for Alternatives to Pesticides
Caroline Cox
Editor, Journal of Pesticide Reform
PO Box 1393
Eugene OR 97440-1393

Washington Toxics Coalition
Philip Dickey, PhD
Staff Scientist
4649 Sunnyside Ave N
Seattle, WA 98103

Coalition for Health, Environment and Economic Rights
Tony Tweedale, Secretary
404 E. Spruce St. #2
Missoula, MT 59802

Cancer Prevention Coalition
Donald L. Hassig, Director
c/o University of Illinois at Chicago
School of Public Health, MC 922
2121 West Taylor Street
Chicago, IL 60612

The Breast Cancer Fund
Janet Nudelman. Director of Program
2107 O'Farrell Street
San Francisco, CA 94115-3419

Alliance for Healthy Homes
Don Ryan, Director
227Mass Av NE #200
Washington, DC 20002

Farmworker Justice Fund, Inc.
Shelley Davis, Co-Executive Director
1010 Vermont Avenue N.W., Suite 915
Washington, D.C. 20005

Agricultural Resources Center
Fawn Pattison, Executive Director
206 New Bern Place
Raleigh, NC 27601

Institute for Agriculture and Trade Policy
David Wallinga, MD, MPA
Co-director, Food and Health Program
2105 First Avenue South
Minneapolis, MN 55404

Roseland Organic Farms
Merrill Clark
27427 M-60 West
Cassopolis, MI 49031

Safer Pest Control Project
Rachel Rosenberg, Executive Director
25 E. Washington Ste. 1515
Chicago, IL 60602-1849

Defenders of Wildlife
Caroline Kennedy
Director of Conservation Initiatives
1130 17th Street, NW
Washington, DC 20036

California Safe Schools
Robina Suwol, Director
5925 Tobias Avenue
Van Nuys, CA 91411

Advocates for Environmental Human Rights
Monique Harden, Co-Director & Attorney
1050 South Jefferson Davis Pkwy.# 333
New Orleans, LA 70125

Californians for Alternatives to Toxics
Patty Clary, Director
315 P Street
Eureka, CA 95501

New York Public Interest Research Group
Laura Haight, Senior Environmental Associate
107 Washington Avenue
Albany, NY 12210

New Jersey Environmental Federation
Jane Nogaki, Pesticide Program
223 Park Avenue
Marlton, NJ 08053

Wyoming Outdoor Council
Steve Jones, Program Attorney
262 Lincoln St.
Lander, WY 82520

Alaska Community Action on Toxics
Pamela K. Miller, Director
505 West Northern Lights Blvd # 205
Anchorage, Alaska 99503

Ecology Center
Tracey Easthope, MPH
Director, Environmental Health Project
117 N. Division
Ann Arbor, MI 48104

Citizens' Environmental Coalition
Kathleen A. Curtis, Executive Director
33 Central Avenue third floor
Albany, New York 12210

Environmental Research Foundation
Peter Montague, Director
P.O. Box 160
New Brunswick, N.J. 08903

Clean Water Action
Bob Wendelgass, PA State Director
100 N. 17th Street, 9th Floor
Philadelphia PA 19103

Toxics Action Center
Matthew L. Wilson, Director
44 Winter Street
Boston MA 02108

Informed Choices
Nancy Hirschfeld, Director
1301 Howze Beach Road
Slidell, LA 70458

National Center for Environmental Health Strategies, Inc.
Mary Lamielle, Executive Director
1100 Rural Avenue
Voorhees, New Jersey 08043

The Coalition for Alternatives to Pesticides
Rohini Peris
1616 Montarville Street
Saint-Bruno-de-Montarville, Quebec
Canada

Texans for Alternatives to Pesticides
Charlotte Wells, Director
3015 Richmond Ste 270
Houston, TX 77098

Jack B. Richman Environmental Coalition
Marcella Richman, Executive Director
4545 W. Touhy St. Apt. 625
Lincolnwood, IL 60712

Colorado Pesticide Network
Angela Medbery
2205 Meade Street
Denver, CO 80211-5055

Grassroots Coalition
Sue Riedeman, Coordinator
Affiliate of Ecological Health Organization
PO Box 0119
Hebron, Connecticut 06248

Women's Voices for the Earth
Aimee Boulanger, Executive Director
P.O. Box 8743
Missoula, MT 59807

Grassroots Environmental Education
Patti Wood, Director
52 Main Street
Port Washington, NY 11050

No Spray Coalition, Nashville
Rachel Sumner
217 Silo Court
Nashville, TN 37221

Students for Bhopal
Ryan Bodanyi, Coordinator
177 Vinton Street
Providence, RI 02909

Coalition for Environmentally Safe Communities
Sarah Gourde
6642 Fisher Ave
Falls Church, VA 22046

Citizens' Campaign for the Environment
Adrienne Esposito
225A Main Street
Farmingdale, NY 11735

Connecticut Coalition for Environmental Justice
Mark Mitchell MD, MPH, President

APPENDIX A: Carcinogenicity Literature Review

Below we summarize, by category, the published literature--both positive and negative results and include all varying quality of journals. In each category, positive toxicity findings heavily and consistently outweigh the negative findings. In accordance with the Agency's carcinogenicity guidelines--considering weight-of-evidence (epidemiology, experimental, mechanistic; and the quality of peer-review)--we conclude that 2,4-D is clearly at least a Class C carcinogen.

POSITIVE RESULTS: GENERAL

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.[80] We ask that the Agency explicitly justify the Agency's '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:52:19:5485a-5488a). The following abstract, presumably to a review, seems to us a fair summary of our findings in reviewing this published literature:

> Reuber MD. 1983 Dec 1. Carcinogenicity and toxicity of 2,4-dichlorophenoxy-acetic acid. Sci Total Environ 31:203-18. **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 isooctyl ester was carcinogenic for the lymphoreticular system in female mice. 2,4-D and 2,4-dichlorophenol also were promoters of neoplasms of the skin in mice. Male mice given 2,4-D isopropyl ester developed an increased incidence of neoplasms of the lung. 2,4-D also is mutagenic and teratogenic in animals and causes poisoning in animals and human beings.

Given that typical chronic toxicology studies are designed not to search for any toxicity,

[80] International Agency for Research on Cancer 1987 'Chlorophenoxy Herbicides' IARC Monographs:(Sup7):156.http://www-cie.iarc.fr/htdocs/monographs/suppl7/chlorophenoxyherbicides.html, accessed Jan. 2004.

we find evidence on timing of the dose to be an essential element of toxicity. The two studies below find that 2,4-D is not carcinogenic when 2,4-D exposure occurs after weaning, but that it is when the exposure occurs earlier:

> Parfieniuk A, Musiatowicz B, Sulik M. 1993 May 3-10. [Some parameters of Guerin cancer growth after exposure to Pielik (sodium salt of 2,4-dichlorophenoxyacetate)]. Pol Tyg Lek 48:414-6.
> **Abstract:** Herbicide Pielik (sodium 2,4-dichlorophenoxyacetate) was tested with the aid of Guerin cancer animal model in 129 Wistar rats. An effect of this herbicide on the cancer growth dynamic (size and weight of the tumor), its malignancy (lymphatic nodes involvement), tumor-dependent animal cachexia (real body weight), and survival of rats depending on exposure period have been analysed. Aqueous solution of the herbicide was administered to animals of groups II, IV, V, and VI in the dose of 200 mg/kg body weight daily (1/3 LD50). Young rats were exposed to the herbicide during pre- and postnatal period till the death (groups III, IV and VI in the 80th day of life. Exposure to the herbicide was continued. Rats of all groups were sacrificed in the 16th, 20th, and 42nd day after implantation of Guerin cancer. Eight animals of each group were kept alive to assess survival. **Accelerated growth of the tumor was noted in the animals exposed to the herbicide for the prolonged period of time (before and after birth). The same daily dose administered to the animals after weaning and continued** to the 16th, 20th, and 42nd day of tumor development (group IV) **has not significant effect on tumor growth rate**. An increase in the incidence as well as earlier onset of metastases to auxillary and groin lymphatic nodes were seen in group VI in comparison with the control animals (group III).

> 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**.

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 there is so much evidence showing 2,4-D to be carcinogenic to the immune system. The EPA must weigh especially heavy the extensive published literature on cancer that we summarize below.

The studies below indicate 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).

POSITIVE RESULTS: NON-HODGKIN'S LYMPHOMA (NHL)

Hardell L, Eriksson M. Department of Oncology, University Hospital, Orebro, Sweden. Environ Health Perspect. 2003 Nov;111(14):1704-6.

Abstract: Is the decline of the increasing incidence of non-Hodgkin lymphoma (NHL) in Sweden and other countries a result of cancer preventive measures? The yearly age-standardized incidence of NHL increased significantly in Sweden during 1971-1990, for men an average of 3.2% and for women 3.1%. The corresponding figures for 1991-2000 were -0.8% and -0.2%, respectively. A decline of the increasing incidence has also been seen in other countries, such as the United States, Finland, and Denmark. Immunosuppression is one established risk factor for NHL, possibly with interaction with Epstein-Barr virus. Phenoxyacetic acids and chlorophenols, both pesticides, have been associated with NHL. Use of these chemicals was banned in Sweden in 1977 and 1978, respectively. Also, persistent organic pollutants such as polychlorinated biphenyls, hexachlorobenzene, chlordanes, and dioxins have been shown to increase the risk. Exposure of the whole population occurs predominantly through the food chain. Exposure to such chemicals was highest in the 1960s and 1970s. Because of regulation in the 1970s, exposure has declined substantially in the population. The change in incidence of NHL in Sweden and other countries may serve as a good example of how prohibition and limitation of exposure may be reflected in cancer statistics some decades later. PMID: 14594618 [PubMed - indexed for MEDLINE]

Zahm SH, Weisenburger DD, Babbitt PA, Saal RC, Vaught JB, Cantor KP, Blair A. 1990 Sep . A case-control study of non-Hodgkin's lymphoma and the herbicide

2,4-dichlorophenoxyacetic acid (2,4-D) in eastern Nebraska. Epidemiology 1:349-56.
Abstract: To evaluate the role of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) in the development of non-Hodgkin's lymphoma (NHL), we conducted a population-based, case-control study in 66 counties in eastern Nebraska. Telephone interviews were conducted with 201 white men diagnosed with NHL between July 1, 1983, and June 30, 1986, and with 725 controls. There was a 50% excess of NHL among men who mixed or applied 2,4-D (odds ratio [OR] = 1.5; 95% confidence interval = 0.9, 2.5). The risk of NHL increased with the average frequency of use to over threefold for those exposed 20 or more days per year (p for trend = 0.051). Adjusting for use of organophosphate insecticides lowered the risk estimate for frequent users (OR = 1.8), but adjustment for fungicide use increased the risk estimate (OR = 4.5). Simultaneous adjustment for organophosphates and fungicides yielded an OR of 3.1 for farmers who mixed or applied 2,4-D more than 20 days per year. Risk also increased with degree of exposure, as indicated by application method and time spent in contaminated clothing, but not with the number of years of 2,4-D use or failure to use protective equipment. Although other pesticides, especially organophosphate insecticides, may be related to NHL, the risk associated with 2,4-D does not appear to be explained completely by these other exposures.

Cantor KP, Blair A, Everett G, Gibson R, Burmeister LF, Brown LM, Schuman L, Dick FR. 1992. Pesticides and other agicultural risk factors for Non-Hodgkin's lymphoma among men in Iowa and Minnesota. Cancer Res 52:2447-2455.
Abstract: Data from an in-person interview study of 622 white men with newly diagnosed non-Hodgkin's lymphoma and 1245 population-based controls in Iowa and Minnesota were used to measure the risk associated with farming occupation and specific agricultural exposures. Men who ever farmed were at slightly elevated risk of non-Hodgkin's lymphoma (odds ratio = 1.2, 95% confidence interval = 1.0-1.5) that was not linked to specific crops or particular animals. Elevated risks were found, with odds ratio generally 1.5-fold or greater, for personal handling, mixing, or application of several pesticide groups and for individual insecticides, including carbaryl, chlordane, dichlorodiphenyltrichloroethane, diazinon, dichlorvos, lindane, malathion, nicotine and toxaphene. Associations were generally stronger for first use prior to 1965 than more recently, and when protective clothing or equipment was not used. Small risks were associated with the use of the phenoxyacetic acid herbicide 2,4-dichlorophenoxyacetic acid, but the risks did not increase with latency of failure to use protective equipment. Exposure to numerous pesticides poses problems of interpreting risk associated with a particular chemical, and multiple comparisons increase the chances of false-positive findings. In contrast nondifferential exposure misclassification due to inaccurate recall can bias risk estimates toward the null and mask positive associations. In the face of these methodological and statistical issues, the consistency of several finding, both within this study and with observations of others, suggests an important role for several insecticides in the etiology on non-Hodgkin's lymphoma among farmers.

Keywords:

Fontana A, Picoco C, Masala G, Prastaro C, Vineis P. 1998. Incidence rates of lymphomas and environmental measurements of phenoxy herbicides: ecological analysis and case-control study. Arch Environ Health 53:384-387.
Abstract: The authors conducted an ecological study of the distribution of malignant lymphomas in a rice-growing area in northern Italy. They considered data on concentrations of phenoxy herbicides in soil and water and found the highest incidence of non-Hodgkin's lymphoma in subjects who lived in an area where 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid existed in very high concentrations. During 1985-1988, the incidence of non-Hodgkin's lymphoma in males in the most-polluted municipalities was twice as high as was noted for the remaining less-polluted territories. During 1991-1993, non-Hodgkin's lymphoma was higher by 60%. The authors also conducted a population-based case-control study. They found an association between employment of women in rice-growing jobs (particularly as rice weeders) and risk of non-Hodgkin's lymphoma (odds ratio = 1.9; 95% confidence interval = 0.6, 6.0). Work in rice fields was correlated strongly with residence in polluted areas. The authors did not detect an association between area of residence or occupation and incidence of Hodgkin's disease.

Hardell L, Eriksson M. Department of Oncology, Orebro Medical Center, Sweden. 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.
BACKGROUND: The incidence of non-Hodgkin lymphoma (NHL) has increased in most Western countries during the last few decades. Immunodefective conditions are established risk factors. In 1981, the authors reported an increased risk for NHL following exposure to certain pesticides. The current study was designed to further elucidate the importance of phenoxyacetic acids and other pesticides in the etiology of NHL. METHODS: A population-based case-control study in northern and middle Sweden encompassing 442 cases and twice as many controls was performed. Exposure data were ascertained by comprehensive questionnaires, and the questionnaires were supplemented by telephone interviews. In total, 404 cases and 741 controls answered the questionnaire. Univariate and multivariate analyses were performed with the SAS statistical data program. RESULTS: Increased risk for NHL was found for subjects exposed to herbicides (odds ratio [OR], 1.6; 95% confidence interval [CI], 1.0-2.5) and fungicides (OR, 3.7; 95% CI, 1.1-13.0). Among herbicides, the phenoxyacetic acids dominated (OR, 1.5; 95% CI, 0.9-2.4); and, when subclassified, one of these, 4-chloro-2-methyl phenoxyacetic acid (MCPA), turned out to be significantly associated with NHL (OR, 2.7; 95% CI, 1.0-6.9). For several categories of herbicides, it was noted that only exposure during the most recent decades before diagnosis of NHL was associated with an increased risk of NHL. Exposure to impregnating agents and insecticides was, at most, only weakly related to NHL. CONCLUSIONS: Exposure to herbicides in total, including

phenoxyacetic acids, during the decades before NHL diagnosis resulted in increased risk for NHL. Thus, the risk following exposure was related to the latency period. Fungicides also increased the risk for NHL when combined, but this group consisted of several different agents, and few subjects were exposed to each type of fungicide. PMID: 10189142 [PubMed - indexed for MEDLINE]

Hardell L, Eriksson M, Degerman A. 1994 May 1. Exposure to phenoxyacetic acids, chlorophenols, or organic solvents in relation to histopathology, stage, and anatomical localization of non-hodgkins lymphoma. Cancer Res 54:2386-2389.
Abstract: Results on 105 cases with histopathologically confirmed non-Hodgkin's lymphoma (NHL) and 335 controls from a previously published case-control study on malignant lymphoma are presented together with some extended analyses. No occupation was a risk factor for NKL. Exposure to phenoxyacetic acids yielded, in the univariate analysis, an odds ratio of 5.5 with a 95% confidence interval of 2.7-11. Most cases and controls were exposed to a commercial mixture of 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid. Exposure to chlorophenols gave an odds ratio of 4.8 (2.7-8.8) with pentachlorophenol being the most common type. Exposure to organic solvents yielded an odds ratio of 2.4 (1.4-3.9). These results were not significantly changed in the multivariate analysis. Dichlorodiphenyltrichloroethane, asbestos, smoking, and oral snuff were not associated with an increased risk for NHL. The results regarding increased risk for NHL following exposure to phenoxyacetic acids, chlorophenols, or organic solvents were not affected by histopathological type, disease stage, or anatomical site of disease presentation. Median survival was somewhat longer in cases exposed to organic solvents than the rest. This was explained by more prevalent exposure to organic solvents in the group of cases with good prognosis NHL histopathology. [References: 29] Number of References 29

Br J Ind Med. 1981 Feb;38(1):27-33. Soft-tissue sarcomas and exposure to chemical substances: a case-referent study. Eriksson M, Hardell L, Berg NO, Moller T, Axelson O. In 1977 several patients were seen with soft-tissue sarcomas and previous exposure to phenoxy acids. This clinical observation resulted in a cases-referent (case-control) study being undertaken which showed that exposure to phenoxy acids or chlorophenols, which are chemically related, gave a roughly six-fold increase in the risk for this type of tumour. A further case-referent study of soft-tissue sarcomas has now been performed to confirm these earlier findings and also to obtain further information on the effects of different phenoxy acids. This new investigation gave an increase of the same magnitude in the risk for soft-tissue sarcomas after exposure to phenoxy acids or chlorophenols, but this risk related also to exposure to phenoxy acids free from impurities, such as polychlorinated dibenzodioxins and dibenzofurans. PMID: 7470401 [PubMed - indexed for MEDLINE]

McDuffie HH, Pahwa P, McLaughlin JR, Spinelli JJ, Fincham S, Dosman JA, Robson D, Skinnider LF, Choi NW. 2001 Nov. Non-hodgkin's lymphoma and

specific pesticide exposures in men: cross-canada study of pesticides and health. Cancer Epidemiology, Biomarkers & Prevention 10:1155-1163. **Abstract:** Our objective in the study was to investigate the putative associations of specific pesticides with non-Hodgkin's Lymphoma [NHL; International Classification of Diseases, version 9 (ICD-9) 200, 202]. We conducted a Canadian multicenter population-based incident, case (n = 517)-control (n = 1506) study among men in a diversity of occupations using an initial postal questionnaire followed by a telephone interview for those reporting pesticide exposure of 10 h/year or more, and a 15% random sample of the remainder. Adjusted odds ratios (ORs) were computed using conditional logistic regression stratified by the matching variables of age and province of residence, and subsequently adjusted for statistically significant medical variables (history of measles, mumps, cancer, allergy desensitization treatment, and a positive history of cancer in first-degree relatives). We found that among major chemical classes of herbicides, the risk of NHL was statistically significantly increased by exposure to phenoxyherbicides [OR, 1.38; 95% confidence interval (CI), 1.06-1.81] and to dicamba (OR, 1.88; 95% Cl, 1.32-2.68). Exposure to carbamate (OR, 1.92; 95% CI, 1.22-3.04) and to organophosphorus insecticides (OR, 1.73; 95% Cl, 1.27-2.36), amide fungicides, and the fumigant carbon tetrachloride (OR, 2.42; 95% Cl, 1.19-5.14) statistically significantly increased risk. Among individual compounds, in multivariate analyses, the risk of NHL was statistically significantly increased by exposure to the herbicides 2,4-dichlorophenoxyacetic acid (2,4-D; OR, 1.32; 95% CL 1.01-1.73), mecoprop (OR, 2.33; 95% CI, 1.58-3.44), and dicamba (OR, 1.68; 95% CI, 1.00-2.81); to the insecticides malathion (OR, 1.83; 95% Cl, 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% Cl, 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 association of NHL with areas of high soil and water 2,4-d levels vs. low levels]

Weisenburger DD. 1990. Environmental epidemiology of non-Hodgkin's lymphoma in eastern Nebraska. Am J Ind Med 18:303-5.

Abstract: The incidence of non-Hodgkin's lymphoma (NHL) is increased in many counties in eastern Nebraska. Histologic analysis has revealed a twofold increase in the clinically aggressive, diffuse large cell subtype of NHL. To investigate the possible association between NHL and agricultural exposures, a population-based case-control study was conducted in eastern Nebraska in 1985. Telephone interviews were conducted with 201 men having histologically confirmed NHL and 725 controls. Among men, the use of the herbicide 2,4-D was associated with a 50% increased risk of NHL (OR 1.5, 95% CI 0.9, 2.4). Personal exposure to 2,4-D more than 20 days per year increased the risk threefold (OR 3.3, 95% CI 0.5, 22.1). Several classes of insecticides were also associated with increased risk: organophosphates (OR 1.9, 95% CI 1.1, 3.1), carbamates (OR 1.8, 95% CI 1.0, 3.2), and chlorinated hydrocarbons (OR 1.4, 95% CI 0.8, 2.3). As a result of intense agrichemical use, extensive contamination of shallow groundwater by nitrate and atrazine has also occurred in eastern Nebraska. A twofold increased incidence of NHL is present in counties with greater than 20% of the wells contaminated by nitrate (greater than 10 ppm) and in counties with intense fertilizer use. These findings suggest that NHL in eastern Nebraska may be related to the use of pesticides and nitrogen fertilizers.

Wiklund K, Lindefors BM, Holm LE. 1988 Jan. Risk of malignant lymphoma in Swedish agricultural and forestry workers. Br J Ind Med 45:19-24.
Abstract: The risk of malignant lymphoma after possible exposure to phenoxy acid herbicides was studied in 354,620 Swedish men who, according to a national census in 1960, were employed in agriculture or forestry. The cohort was divided into subcohorts according to assumed exposure and compared with 1,725,645 Swedish men having other economic activities. All were followed up in the Cancer-Environment Register between 1961 and 1979. Non-Hodgkin lymphoma was found in 861 men in the study cohort. The relative risk was not significantly increased in any subcohort, did not differ significantly between the subcohorts, and showed no time related increase in the total cohort or any subcohort. Hodgkin's disease was found in 355 men in the study cohort. Relative risks significantly higher than unity were found among fur farming and silviculture workers where the relative risks were 4.45 and 2.26, respectively. All five cases in the former group were engaged in mink farming. A time related rising trend in relative risk was found in the silviculture subcohort. Elsewhere the relative risk did not diverge from unity and no time related trend was discernible.

Zahm SH, Weisenburger DD, Babbitt PA, Saal RC, Vaught JB, Cantor KP, Blair A. 1990. A Case-Control Study of Non-Hodgkin's Lymphoma and the Herbicide 2,4-Dichlorophenoxyacetic Acid (2,4-D) in Eastern Nebraska. Epidemiology 1:349-356.
Abstract: To evaluate the role of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) in the development of non-Hodgkin's lymphoma (NHL), we conducted a population-based, case-control study in 66 counties in eastern Nebraska. Telephone interviews were conducted with 201 white men diagnosed with NHL between July 1, 1983, and June 30, 1986, and with 725 controls. There was a

50% excess of NHL among men who mixed or applied 2,4-D (odds ratio [OR] = 1.5; 95% confidence interval = 0.9, 2.5). The risk of NHL increased with the average frequency of use to over threefold for those exposed 20 or more days per year (p for trend = 0.051). Adjusting for use of organophoshate insecticides lowered the risk estimate (OR = 4.5). simultaneous adjustment for organophosphates and fungicides yielded an OR of 3.1 for farmers who mixed or applied 2,4-D more that 20 days per year. Risk also increased with degree of exposure, as indicated by application method and time spent in contaminated clothing, but not with the number of years of 2,4-D use or failure to use protective equipment. Although other pesticides, especially organophosphate insecticides, may be related to NHL, the risk associated with 2,4-D does not appear to be explained completely by these other exposures.Keywords:

Scand J Work. Environ Health. 1994 Feb;20(1):42-7. Non-Hodgkin's lymphoma and agricultural practices in the prairie provinces of Canada. Morrison HI, Semenciw RM, Wilkins K, Mao Y, Wigle DT. Bureau of Chronic Disease Epidemiology, Laboratory Centre for Disease Control, Health Canada, Ottawa. OBJECTIVES--The aim of this study was to provide an update of a cohort study (1971-1985) that previously reported a significant trend in the risk of non-Hodgkin's lymphoma among male Saskatchewan farm operators according to fuel-oil expenditures and herbicide spraying for farms less than 1000 acres (2570 hectares) by including two additional Canadian prairie provinces, two additional years of follow-up, and data from the 1981 Census of Agriculture. METHODS--Information on farmers from 1971 records of the Census of Agriculture was linked to 1971 records of the Census of Population, to 1981 records of the Census of Agriculture, and to death records. Poisson regression was used to estimate risks according to herbicide spraying and fuel and oil expenditures. RESULTS--The addition of a further two years of follow-up resulted in lower risk estimates associated with herbicide spraying for Saskatchewan. No excess risk was observed between herbicide spraying and non-Hodgkin's lymphoma for Alberta or Manitoba in the 1971 data. However, a significantly increased risk of non-Hodgkin's lymphoma according to acres sprayed with herbicides was observed for the three provinces combined when the herbicide spraying data from the 1981 Census of Agriculture was used [> or = 380 acres (> or = 939 hectares) sprayed, rate ratio 2.11, 95% confidence interval 1.1-3.9]. CONCLUSIONS--Although the current results are not entirely consistent with the original Saskatchewan analysis, they support the overall finding of an association between herbicides and risk of fatal non-Hodgkins lymphoma. Prospective cohort studies are needed to overcome the limitations of existing epidemiologic studies. PMID: 8016598 [PubMed - indexed for MEDLINE

Med Lav. 1990 Nov-Dec;81(6):499-505. Mortality study of Canadian male farm operators: cancer mortality and agricultural practices in Saskatchewan. Ritter L, Wigle DT, Semenciw RM, Wilkins K, Riedel D, Mao Y. Health Protection Branch, Health and Welfare Canada, Ottawa, Ontario.
OBJECTIVE: 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.

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. Journal of the National Cancer Institute 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.

Sternberg SS. 1992 Feb 19. Canine malignant lymphoma and 2,4-dichlorophenoxyacetic acid herbicides. J Natl Cancer Inst 84:271.

In addition, the following study strongly supports 2,4-D carcinogenicty to pets in close contact with it. Such exposure is further proven by the study after it.

J Am Vet Med Assoc. 2004 Apr 15;224(8):1290-7. Herbicide exposure and the risk of transitional cell carcinoma of the urinary bladder in Scottish Terriers.
Glickman LT, Raghavan M, Knapp DW, Bonney PL, Dawson MH. Department of Veterinary Pathobiology, School of Veterinary Medicine, Purdue University, West Lafayette, IN 47907-2027, USA.
OBJECTIVE: To determine whether exposure to lawn or garden chemicals was associated with an increased risk of transitional cell carcinoma (TCC) of the urinary bladder in Scottish Terriers. DESIGN: Case-control study. ANIMALS: 83 Scottish Terriers with TCC (cases) and 83 Scottish Terriers with other health-related conditions (controls). PROCEDURE: Owners of study dogs completed a written questionnaire pertaining to exposure to lawn or garden chemicals during the year prior to diagnosis of TCC for case dogs and during a comparable period for control dogs. RESULTS: The risk of TCC was significantly increased among dogs exposed to lawns or gardens treated with both herbicides and insecticides (odds ratio [OR], 7.19) or with herbicides alone (OR, 3.62), but not among dogs exposed to lawns or gardens treated with insecticides alone (OR, 1.62), compared with dogs exposed to untreated lawns. Exposure to lawns or gardens treated with phenoxy herbicides (OR, 4.42) was associated with an increased risk of TCC, compared with exposure to untreated lawns or gardens, but exposure to lawns or gardens treated with nonphenoxy herbicides (OR, 3.49) was not significantly associated with risk of TCC. CONCLUSIONS AND CLINICAL RELEVANCE: Results suggest that exposure to lawns or gardens treated with herbicides was associated with an increased risk of TCC in Scottish Terriers. Until additional studies are performed to prove or disprove a cause-and-effect relationship, owners of Scottish Terriers should minimize their dogs' access to lawns or gardens treated with phenoxy herbicides. PMID: 15112777 [PubMed - indexed for MEDLINE]

Reynolds PM, Reif JS, Ramsdell HS, Tessari JD. 1994 Apr-May. Canine exposure to herbicide-treated lawns and urinary excretion of 2,4-dichlorophenoxyacetic acid. Cancer Epidemiol Biomarkers Prev 3:233-7.
Abstract: A recent study by Hayes et al. (J. Natl. Cancer. Inst., 83: 1226-1231, 1991) found an increased risk of malignant lymphoma associated with exposure to 2,4-dichlorophenoxyacetic acid (2,4-D) in pet dogs. We conducted a study to determine the extent to which dogs absorb and excrete 2,4-D in urine after contact with treated lawns under natural conditions. Among 44 dogs potentially exposed to 2,4-D-treated lawns an average of 10.9 days after application, 2,4-D concentrations greater than or equal to 10.0 micrograms/l were found in 33 dogs (75%) and concentrations of > or = 50 micrograms/l were found in 17 (39%).

Among 15 dogs with no known exposure to a 2,4-D-treated lawn in the previous 42 days, 4 (27%) had evidence of 2,4-D in urine, 1 at a concentration of > or = 50 micrograms/l. The odds ratio for the association between exposure to a 2,4-D-treated lawn and the detection of > or = 50 micrograms/l 2,4-D in urine was 8.8 (95% confidence interval, 1.4-56.2). Dogs exposed to lawns treated within 7 days before urine collection were more than 50 times as likely to have 2,4-D at concentrations > or = 50 micrograms/l than dogs with exposure to a lawn treated more than 1 week previously (odds ratio = 56.0; 95% confidence interval, 10.0-312.2). The highest mean concentration of 2,4-D in urine (21.3 mg/l) was found in dogs sampled within 2 days after application of the herbicide.(ABSTRACT TRUNCATED AT 250 WORDS)

POSITIVE RESULTS: MULTIPLE MYELOMA

Am J Ind Med. 1992;22(3):305-12. Malignant lymphoproliferative diseases in occupations with potential exposure to phenoxyacetic acids or dioxins: a register-based study. Eriksson M, Hardell L, Malker H, Weiner J. Department of Oncology, University Hospital, Umea, Sweden. The Swedish Cancer Environment Register (CER) is a linkage of census data (e.g., on occupations) with the Swedish Cancer Register. It has been used in different studies to generate hypotheses on occupational risk factors for malignant tumors. In this study the risk for malignant lymphoma and multiple myeloma in occupations with potential exposure to phenoxyacetic acids or other related substances were investigated. An increased standardized incidence ratio (SIR) of 1.3 for multiple myeloma was verified in farmers (no. of cases = 335). This finding applied to both sexes, and the SIR increased over successive time periods. Regarding malignant lymphoma an increased SIR of 1.2 was found in farmers (no. = 227) for the latest time period studied (i.e. 1979-1984). When non-Hodgkin's lymphoma was studied separately, an increased risk (SIR = 1.2) was found only in carpenters (no. = 149), whereas for Hodgkin's disease, sawmill workers (no. = 10) had an increased SIR of 2.1. Physicians also had an elevated risk for malignant lymphoma. A major shortcoming in register studies such as CER is that no individual exposure data on different agents are available. Lack of an association between an occupation and a specific malignant disease, therefore, may not be taken as evidence that persons within that occupation are not at increased risk for that disease. PMID: 1519615 [PubMed - indexed for MEDLINE]

POSITIVE RESULTS: LEUKEMIA

Brown LM, Blair A, Gibson R, Everett GD, Cantor KP, Schuman LM, Burmeister LF, Van Lier SF, Dick F. 1990 Oct 15. Pesticide exposures and other agricultural risk factors for leukemia among men in Iowa and Minnesota. Cancer Res 50:6585-91.
Abstract: Mortality surveys and death certificate studies have suggested an association between leukemia and farming. To investigate whether exposure to

carcinogens in an agricultural setting is related to risk of leukemia, the authors conducted a population-based case-control interview study of 578 white men with leukemia and 1245 controls living in Iowa and Minnesota. Consistent with recent mortality studies, there were slight, but significant, elevations in risk for all leukemia [odds ratio (OR) 1.2] and chronic lymphocytic leukemia (OR 1.4) for farmers compared to nonfarmers. There were no significant associations with leukemia for exposure to specific fungicides, herbicides (including 2,4-D and 2,4,5-T), or crop insecticides. However, significantly elevated risks for leukemia of greater than or equal to 2.0 were seen for exposure to specific animal insecticides including the organophosphates crotoxyphos (OR 11.1), dichlorvos (OR 2.0), and famphur (OR 2.2) and the natural product pyrethrins (OR 3.7) and the chlorinated hydrocarbon methoxychlor (OR 2.2). There were also smaller, but significant, risks associated with exposure to nicotine (OR 1.6) and DDT (OR 1.3). This finding of elevated risks for insecticides used on animals deserves further evaluation.

Schreinemachers DM. 2000 Sep. Cancer mortality in four northern wheat-producing states. Environ Health Perspect 108:873-881.
Abstract: Chlorophenoxy herbicides are used both in cereal grain agriculture and in nonagricultural settings such as right-of-ways, lawns, and parks. Minnesota, North Dakota, South Dakota, and Montana grow most of the spring and durum wheat produced in the United States. More than 90% of spring and durum wheat is treated with chlorophenoxy herbicides, in contrast to treatment of approximately 30% of winter wheat. In this ecologic study I used wheat acreage as a surrogate for exposure to chlorophenoxy herbicides. I investigated the association of chlorophenoxy herbicides with cancer mortality during 1980-1989 for selected counties based on level of agriculture (greater than or equal to 20%) and rural population (greater than or equal to 50%). Age-standardized cancer mortality rates were determined for grouped counties based on tertiles of wheat acreage per county or for individual counties for frequently occurring cancers. The cancer sites that showed positive trends of increasing cancer mortality with increasing wheat acreage were esophagus, stomach, rectum, pancreas, larynx, prostate, kidney and meter, brain, thyroid, bone, and all cancers (men) and oral cavity and tongue, esophagus, stomach, liver and gall bladder and bile ducts, pancreas, cervix, ovary, bladder, and other urinary organs, and all cancers (women). Rare cancers in men and women and cancers in boys and girls were studied by comparing counties above and below the median of wheat acreage per county. There was increased mortality for cancer of the nose and eye in both men and women, brain and leukemia in both boys and girls, and all cancers in boys. These results suggest an association between cancer mortality and wheat acreage in counties of these four states. [References: 52] Number of References 52

Swaen GMH, van Amelsvoort LGPM, Slangen JJM, Mohren DCL. 2004 May. Cancer mortality in a cohort of licensed herbicide applicators. International Archives of Occupational & Environmental Health 77:293-295.
Abstract: Objectives. In order to expand our knowledge on the possible long-term health effects of exposure to herbicides, we updated the follow-up of a cohort of 1,341 licensed

herbicide applicators in the Netherlands. The earlier report indicated that there might be an increased risk for multiple myeloma in this group. Although that finding was statistically significant, the result was based on a small number of cases. Methods. We expanded the follow-up from 1 January 1988 to 1 January 2001, which added 13 years to the follow-up. We now report on the causes of death of 196 exposed workers. Results. Our findings indicate that licensed herbicide applicators were at an increased risk for skin cancer mortality [standardized mortality ratio (SMR)=357.4, 95% confidence interval (CI) 115.1-827.0]. It is not clear if this excess of skin cancer should be attributed to herbicide exposure or to excess exposure to sunlight. [References: 12] Number of References 12

POSITIVE RESULTS: SOFT TISSUE SARCOMAS (STS); BRAIN; AND OTHER NON-IMMUNE CANCERS

Brain Cancer

The authors of this un-translated paper consistently correlate phenoxy herbicides with cancers.

> Lakartidningen. 1997 Feb 26;94(9):728-31. [Increased incidence of brain tumors. A study of Swedish children and adolescents aged 0-19] [Article in Swedish] Hardell L, Tondel M, Flodin U, Skoldestig A, Axelson O, Jakobsson S, Eriksson M, Carlsson G. Onkologiska kliniken, Regionsjukhuset, Orebro. PMID: 9091748 [PubMed - indexed for MEDLINE]

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']

> 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:

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 [THIS STUDY INDIRECTLY SUPPORTS EPA'S FINDINGS OF ASTROCYTOMAS]---

POSITIVE RESULTS: STS AND UNSPECIFIED CANCERS

JAMA. 1986 Sep 5;256(9):1141-7. Erratum in: * JAMA 1986 Dec 26;256(24):3351.
Agricultural herbicide use and risk of lymphoma and soft-tissue sarcoma. Hoar SK, Blair A, Holmes FF, Boysen CD, Robel RJ, Hoover R, Fraumeni JF Jr.
A population-based case-control study of soft-tissue sarcoma (STS), Hodgkin's disease (HD), and non-Hodgkin's lymphoma (NHL) in Kansas found farm herbicide use to be associated with NHL (odds ratio [OR], 1.6; 95% confidence interval [CI], 0.9, 2.6). Relative risk of NHL increased significantly with number of days of herbicide exposure per year and latency. Men exposed to herbicides more than 20 days per year had a sixfold increased risk of NHL (OR, 6.0; 95% CI, 1.9, 19.5) relative to nonfarmers. Frequent users who mixed or applied the herbicides themselves had an OR of 8.0 (95% CI, 2.3, 27.9) for NHL. Excesses were associated with use of phenoxyacetic acid herbicides, specifically 2,4-dichlorophenoxyacetic acid. Neither STS nor HD was associated with pesticide exposure. This study confirms the reports from Sweden and several US states that

NHL is associated with farm herbicide use, especially phenoxyacetic acids. It does not confirm the case-control studies or the cohort studies of pesticide manufacturers and Vietnam veterans linking herbicides to STS or HD.
[THE ERRATUM IS NO MORE THAN A CHANGE IN THE TITLE OF THE MAIN RESULTS TABLE.]

Hardell L, Sandstrom A. 1979 Jun. Case-control study: soft-tissue sarcomas and exposure to phenoxyacetic acids or chlorophenols. Br J Cancer 39:711-7. **Abstract:** In 1977 a number of patients with soft-tissue sarcomas and previous exposure to phenoxyacetic acids were described. Following from these observations a matched case-control study was made. Exposure to chlorophenols was also included in this study. The results showed that exposure to phenoxyacetic acids or chlorophenols gave an approximately 6-fold increase in the risk for this type of tumour. It was not possible to determine, however, whether the carcinogenic effect was exerted by these compounds or by impurities such as chlorinated dibenzodioxins and dibenzofurans that in almost all cases were part of the commercial preparations.

J Natl Cancer Inst. 1990 Mar 21;82(6):486-90. Comment in: * J Natl Cancer Inst. 1990 Nov 21;82(22):1785-6. Exposure to dioxins as a risk factor for soft tissue sarcoma: a population-based case-control study. Eriksson M, Hardell L, Adami HO. Department of Oncology, University Hospital, Umea, Sweden.
In a case-control study including 237 cases with soft tissue sarcoma and 237 controls, previous jobs and exposures to different agents, including pesticides, were assessed. Exposure to phenoxyacetic acids or chlorophenols gave a statistically significant increased rate ratio (RR) of 1.80 [95% confidence interval (CI) = 1.02-3.18] for soft tissue sarcoma. Exposure to phenoxyacetic acids of all types gave a nonsignificantly increased RR of 1.34 (95% CI = 0.70-2.56). During the 1950s, exposure to 2,4,5-trichlorophenoxyacetic acid gave a threefold significantly increased risk. High-grade exposure to chlorophenols, which are also contaminated by dioxins, gave an RR of 5.25 (95% CI = 1.69-16.34). The increased risk was thus attributed to dioxin-contaminated phenoxyacetic acids or chlorophenols that gave an RR of 2.43 (95% CI = 1.30-4.54). PMID: 2313720 [PubMed - indexed for MEDLINE]

Cancer. 1988 Aug 1;62(3):652-6. The association between soft tissue sarcomas and exposure to phenoxyacetic acids. A new case-referent study. Hardell L, Eriksson M. Department of Oncology, University Hospital, Umea, Sweden.
A case-referent study on soft tissue sarcomas (STS) was conducted, to see if previous findings regarding an association between exposure to phenoxyacetic acids or chlorophenols and this tumor type could be reproduced. Fifty-five male STS patients were thereby compared with 220 living and 110 dead population-based referents. Furthermore, another referent group consisting of 190 patients with another type of malignant disease was used in order to evaluate any influence of recall bias on the results. To obtain information about exposure to the studied chemicals, as well as about any other exposures that might be of interest,

questionnaires were used, and if necessary these were completed over the phone by an interviewer who had no information regarding case-referent status. All analysis and interpretation of exposure data were done in a blinded manner. Exposure to phenoxyacetic acids gave a roughly three-fold increased risk for STS, thereby confirming previous findings, whereas exposure to chlorophenols was not associated with STS in this study. PMID: 3390800 [PubMed - indexed for MEDLINE]

Lakartidningen. 1981 Aug 19;78(34):2862-3. [Phenoxyacetic acid, chlorphenols and cancer] [Article in Swedish] Hardell L, Eriksson M. PMID: 7321672 [PubMed - indexed for MEDLINE]

Br J Cancer. 1981 Feb;43(2):169-76. Malignant lymphoma and exposure to chemicals, especially organic solvents, chlorophenols and phenoxy acids: a case-control study. Hardell L, Eriksson M, Lenner P, Lundgren E.
A number of men with malignant lymphoma of the histiocytic type and previous exposure to phenoxy acids or chlorophenols were observed and reported in 1979. A matched case-control study has therefore been performed with cases of malignant lymphoma (Hodgkin's disease and non-Hodgkin lymphoma). This study included 169 cases and 338 controls. The results indicate that exposure to phenoxy acids, chlorophenols, and organic solvents may be a causative factor in malignant lymphoma. Combined exposure of these chemicals seemed to increase the risk. Exposure to various other agents was not obviously different in cases and in controls. PMID: 7470379 [PubMed - indexed for MEDLINE]

Lakartidningen. 1979 Oct 31;76(44):3872-5. [Case- control study of malignant mesenchymal soft tissue tumors and exposure to chemical substances] [Article in Swedish] Eriksson M, Hardell L, Berg NO, Moller T, Axelson O. PMID: 529930 [PubMed - indexed for MEDLINE]

Kogevinas M, Kauppinen T, Winkelmann R, Becher H, Bertazzi PA, Buenodemesquita HB, Coggon D, Green L, Johnson E, Littorin M, Lynge E, Marlow DA, Mathews JD, Neuberger M, Benn T, Pannett B, Pearce N, Saracci R. 1995. Soft tissue sarcoma and non-hodgkins lymphoma in workers exposed to phenoxy herbicides, chlorophenols, and dioxins - TWO nested case-control studies. Epidemiology 6:396-402.
Abstract: We examined the effect of exposure to chemicals present in the production and spraying of phenoxy herbicides or chlorophenols in two nested case-control studies of soft tissue sarcoma and non-Hodgkin's lymphoma. Eleven sarcoma and 32 lymphoma cases occurring within an international cohere were matched for age, sex, and country of residence with 55 and 158 controls, respectively. Exposures to 21 chemicals or mixtures were estimated by three industrial hygienists who were blind to the subject's case-control status. Excess risk of soft tissue sarcoma was associated with exposure to any phenoxy herbicide [odds ratio (OR) = 10.3; 95% confidence interval (CI) 1.2-91] and to each of the three major classes of phenoxy herbicides (2,4-dichlorophenoxyacetic acid, 2,4,5-

trichlorophenoxyacetic acid, and 4-chloro-2-methylphenoxyacetic acid), to any polychlorinated dibenzodioxin or furan (OR = 5.6; 95% CI = 1.1-28), and to 2,3,7,8-tetrachlorodibenzo-p-dioxin (OR = 5.2; 95% CI = 0.85-32). Sarcoma risk was not associated with exposure to raw materials or other process chemicals. In the non-Hodgkin's lymphoma study, associations were generally weaker than those found in the study on sarcoma. These findings indicate that workers exposed to phenoxy herbicides and their contaminants are at a higher risk of soft tissue sarcoma.

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.
OBJECTIVE: 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 (Cl) 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

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 – not even in one

of the few less rigorous quality journals.

NEGATIVE RESULTS: NHL AND ASTROCYTOMA

Bond GG, Wetterstroem NH, Roush GJ, McLaren EA, Lipps TE, Cook RR. 1988 Feb. Cause specific mortality among employees engaged in the manufacture, formulation, or packaging of 2,4-dichlorophenoxyacetic acid and related salts. Br J Ind Med 45:98-105.
Abstract: Mortality is reported to the end of 1982 for 878 chemical workers potentially exposed to 2,4-dichlorophenoxyacetic acid (2,4-D) at any time between 1945 and 1983. Observed mortality was compared with expected levels based on adjusted rates for United States white men and for other male employees from this manufacturing location who were not exposed to 2,4-D. Because of a recently reported increased incidence of astrocytomas in male rats fed the highest dose level of 2,4-D, special attention was given to deaths from brain neoplasms in the cohort. None was observed. The absence of an increased risk of brain cancer in people exposed to 2,4-D is supported by studies of other exposed populations and those studies are briefly reviewed. Moreover, in the present study, analyses by production area, duration of exposure, and cumulative dose showed no patterns suggestive of a causal association between 2,4-D exposure and any other particular cause of death.

NEGATIVE RESULTS: NHL

Bloemen LJ, Mandel JS, Bond GG, Pollock AF, Vitek RP, Cook RR. 1993 Dec. An update of mortality among chemical workers potentially exposed to the herbicide 2,4-dichlorophenoxyacetic acid and its derivatives. J Occup Med 35:1208-12.
Abstract: Four years of additional mortality follow-up through 1986 are reported for a previously studied cohort of 878 chemical workers who were potentially exposed to 2,4-dichlorophenoxyacetic acid (2,4-D) and its derivatives between 1945 and 1983. Observed mortality was compared with expected levels based on death rates of the US population and of 36,804 "unexposed" workers from the same manufacturing location. Non-Hodgkin's lymphoma (NHL) was a particular focus of the study because of a suggested association with 2,4-D exposure in some case-control studies. For the total observation period, the standardized mortality ratios for all causes and for malignant neoplasms were 92 and 91, respectively. Analyses using the internal comparison group yielded virtually identical results. The initial study had found two deaths from NHL, both of which occurred under circumstances (ie, short latency and modest exposure) which made it less plausible that they were related to 2,4-D exposure. No new deaths from NHL were observed in the extended follow-up period and mortality for this cause showed a nonstatistically significant excess (standardized mortality ratio, 196; 95% confidence interval 24 to 708) for the total observation period. Analyses by production area, and by two different measures of exposure, combined with two different approaches to account for latency, did not show patterns suggestive of a causal relationship between exposure to

2,4-D or its derivatives and any particular cause of death.

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

Wiklund K, Holm LE. 1986 Feb. Soft tissue sarcoma risk in Swedish agricultural and forestry workers. J Natl Cancer Inst 76:229-34.
Abstract: The risk of soft tissue sarcoma following possible exposure to phenoxy acid herbicides was studied in 354,620 Swedish men, who were employed in agriculture or forestry according to a national census in 1960. This cohort was further divided into six subcohorts, on assumed exposure to phenoxy acid herbicides. The most commonly used phenoxy acid in Sweden was (4-chloro-2-methylphenoxy)acetic acid (CAS: 94-74-6). The reference cohort encompassed 1,725,845 Swedish men employed in other industries. All persons were followed up in the cancer-environment register during the period 1961-79. A total of 331 cases of soft tissue sarcomas was observed in the study cohort and there were 1,508 cases in the reference group [relative risk (RR), 0.9; 95% confidence interval, 0.8-1.0]. No subcohort of agricultural or forestry workers showed any significantly increased RR, nor was there any significant difference in RR between the subcohorts. Despite the greatly increased use of phenoxy acid herbicides from 1947 to 1970, no time-related increase in the RR of soft tissue sarcoma was found in the total cohort or in any of the subcohorts.

NEGATIVE RESULTS: NHL ANALOGUE: CANINE MALIGNANT LYMPHOMA (CML)

Edwards MD, Pazzi KA, Gumerlock PH, Madewell BR. 1993. C-n-ras is activated infrequently in canine malignant lymphoma. Toxicol Pathol 21:288-291. **Abstract:** Activated c-N-ras alleles have been detected in human lymphoma specimens. The aim of the present study was to determine the frequency of c-N-ras mutational activation in canine malignant lymphoma. DNA was isolated from 28 canine malignant lymphoma specimens collected from 28 separate dogs and examined for c-N-ras mutations by polymerase chain reaction amplification and direct sequencing. The tumors were naturally occurring and derived from 20 dogs with known exposures to the phenoxy herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) and from 8 dogs with no known exposure to the herbicide. An oncogenically activating mutation was found in 1 dog without known 2,4-D exposure. The mutation was a 13th codon, second position transition that would result in a glycine-to-aspartate amino acid substitution. The results of this study demonstrate that, similar to the human, c-N-ras mutations are uncommon in dogs with malignant lymphoma and that there is no association between 2,4-D exposure and activation of c-N-ras in the dog.

Gavazza A, Presciuttini S, Barale R, Lubas G, Gugliucci B. 2001 May-2001 Jun 30. Association between canine malignant lymphoma, living in industrial areas, and use of chemicals by dog owners. J Vet Intern Med 15:190-195. **Abstract:** A case-control study was carried out to determine whether residential exposure to environmental pollutants increased risk for canine lymphoma in pet dugs. One hundred one cases with cyctologically or histologically confirmed lymphoma diagnosed at a veterinary teaching hospital between the middle of 1996 and the middle of 1998 were examined. Controls were obtained by choosing twice the number of dogs without neoplastic disease. with overlapping distributions of province of residence, age, sex. and breed. Information regarding animal management. residence type, professional or hobby use of chemicals by owners, and treatment with herbicides or other pesticides in the area li frequently visited by the dogs was obtained with a multiple-choice questionnaire by telephone interview. Two variables were positively and independently associated with the disease. namely residency in industrial areas (odds ratio [OR]: = 8.5: 95% confidence interval [CI]. 2.3-30.9) and use of chemicals by owners, specifically paints or solvents (OR = 4.6: 95% CI. 1.7-12.6). A significantly lower value of the mean apr of disease onset was found in the group of dogs at risk in comparison with the group of all other dogs (6.1 +/- 0.4 years, n = 36 versus 7.5 +/- 0.4 years. n = 65, respectively; P = .008). Variables describing animal care and pesticide use were either not associated with the disease or were uninformative. We suggest that canine lymphoma may be considered a sentinel of potentially hazardous situations for humans, because of the relatively short latency between exposure and disease onset. [References: 27] Number of References 27

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

O'Brien DJ, Kaneene JB, Getis A, Lloyd JW, Swanson GM, Leader RW. 2000 Nov 16. Spatial and temporal comparison of selected cancers in dogs and humans, michigan, usa, 1964-1994. Prev Vet Med 47:187-204.
Abstract: Our aim was to investigate the geographic acid time distributions of some biologically similar neoplasms in dogs and humans living in Michigan, USA, between 1964 and 1994. Our objective was to describe and compare the patterns of cancer in the two species while assessing the strength and dependence of those patterns. In this retrospective, registry-based study, histologically confirmed incident human and canine cancer cases were mapped, and second-order (K function) spatial analysis and one-dimensional nearest neighbor temporal analysis were performed on residence addresses and dares of hospital discharge/diagnosis. Included in the study were all 528 incident cases of canine lymphosarcoma, mammary adenocarcinoma, melanoma and spindle-cell sarcomas diagnosed at a veterinary teaching hospital between 1964 and 1994 having residence addresses in Ingham, Oakland, and Wayne Counties; and a stratified random sample of 913 incident human cases of comparable cancers diagnosed during the same time period from the same counties. Results suggest that processes determining spatial aggregation of cases in dogs and humans were not independent of each other, did not act uniformly over different geographic areas, operated at spatial scales <2000 m regardless of species, and tend to act upon dogs more strongly at shorter distances than on humans. Little evidence of interspecies concurrence of temporal clustering was found. (C) 2000 Elsevier Science B.V. All rights reserved. [References: 57] Number of References 57

====

MECHANISMS OF CANCER

Mutagenicity (DNA/Chromosome 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 latter especially leads to cancer, as both cancer and some cell-cycle disruption involve uncontrolled cell replication.

The weight-of-evidence in this subset is notably in opposition to the Agency's 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 with no standards on conflicts of interest. In contrast, we found 15 published papers showing that 2,4-D is mutagenic (all in journals with quality peer review).

POSITIVE RESULTS: MUTAGENIC

Arias E. 2003 Jul. Sister chromatid exchange induction by the herbicide 2,4-dichlorophenoxyacetic acid in chick embryos. Ecotoxicology & Environmental Safety 55:338-343.
Abstract: As genetic damage may result from exposure to agricultural chemicals, it seemed appropriate to assess the genotoxic potential of 2,4-dichlorophenoxyacetic acid (2,4-D), a widely used broad-leaf herbicide, using a test system that may provide some indications on the genetic risk to animal species in the wild. In the present study, sister chromatid exchange (SCE) induction and cell cycle kinetics alterations by 2,4-D in 4-day old chick embryos were evaluated. Both a commercial herbicide formulation containing 37% 2,4-D isooctyl ester as active ingredient and pure 2,4-D were tested. Chick embryos were treated with 0, 0.5, 1, 2, or 4 mg 2,4-D. Test solutions were applied to the inner shell membrane on day 0 of incubation. Either commercial formulation or pure 2,4-D induced a dose-related increase in SCE frequency over the concentration range from 0 to 4 mg/embryo. Significantly higher SCE frequency was seen for the 4-mg group of embryos treated with the commercial product. A slightly higher SCE value was observed for the vehicle group (acetone-treated embryos) compared with the negative controls (untreated embryos). Significant inhibition of cell cycle progression was evident in both experimental groups and was generally dose related. The extent of changes in cell kinetics was similar in both groups, although somewhat more marked in the group treated with pure 2,4-D. The present findings corroborate the positive results from recent in vivo rodent studies. [References: 36] Number of References 36

Ateeq B, Farah MA, Ali MN, Ahmad W. 2002 Feb 15. Clastogenicity of pentachlorophenol, 2,4-d and butachlor evaluated by allium root tip test. Mutation Research-Genetic Toxicology & Environmental Mutagenesis 514:105-113.
Abstract: The meristematic mitotic cells of Allium cepa is an efficient

cytogenetic material for chromosome aberration assay on environmental pollutants. For assessing genotoxicity of pentachlorophenol (PCP), 2,4-dichlorophenoxyacetic acid (2,4-D) and 2-chloro-2,6-diethyl-N-(butoxymethyl) acetanilide (butachlor), 50% effective concentration (EC50), c-mitosis, stickiness, chromosome breaks and mitotic index (MI) were used as endpoints of genotoxicity. EC50 values for PCP and butachlor are 0.73 and 5.13 ppm, respectively. 2,4-D evidently induced morphological changes at higher concentrations. Some changes like crochet hooks, c-tumours and broken roots were unique to 2,4-D at 5-20 ppm. No such abnormalities were found in PCP and butachlor treated groups, however, root deteriorated and degenerated at higher concentrations (<3 ppm) in PCP. MI in 2,4-D showed a low average of 14.32% followed by PCP (19.53%), while in butachlor it was recorded 71.6%, which is near to the control value. All chemicals induced chromosome aberrations at statistically significant level. The highest chromosome aberration frequency (11.90%) was recorded in PCP at 3 ppm. Large number of c-mitotic anaphases indicated that butachlor acts as potent spindle inhibitor, whereas, breaks, bridges, stickiness and laggards were most frequently found in PCP showing that it is a potent clastogen. (C) 2002 Elsevier Science B.V. All rights reserved. [References: 30] Number of References 30

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. **OBJECTIVE:** Evaluate peripheral blood lymphocyte proliferation (replicative index:RI) and micronuclei frequency (MF) among 2,4-D herbicide applicators. METHODS: Twelve applicators spraying only 2,4-D provided a blood and urine specimen upon enrollment, several urine samples during the spraying season, and a blood specimen at the study's end. Nine controls provided blood and urine specimens upon enrollment and at the study's end. Gas chromatography/tandem mass spectroscopy determined urinary 2,4-D levels and standard in-vitro assays determined RI and MF scores. Applicator RI and MF were compared before and after spraying and with controls. RESULTS: Applicators contributed 45 urine specimens with concentrations ranging from 1.0 to 1700 (microg 2,4-D/g creatinine/L urine) that logarithmically (In) increased as spraying time increased. Applicator RI increased after spraying ($p = 0.016$), independent of tobacco and alcohol use, and demonstrated a weak dose-response with increasing urinary 2,4-D levels ($p = 0.15$). Among 2,4-D applicators, pre-exposure complete blood counts and lymphocyte immunophenotypes were not significantly different from post-exposure measurements. CONCLUSION: Urinary 2,4-D concentration, an exposure biomarker, may be associated with lymphocyte replicative index, a cell proliferation biomarker.

Holland NT, Duramad P, Rothman N, Figgs LW, Blair A, Hubbard A, Smith MT. 2002 Nov 26. Micronucleus frequency and proliferation in human lymphocytes after exposure to herbicide 2,4-dichlorophenoxyacetic acid in vitro and in vivo.

Mutation Research-Genetic Toxicology & Environmental Mutagenesis 521:165-178.
Abstract: Widespread use of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) and its association with non-Hodgkin's lymphoma (NHL) and other cancers has raised public concern. Here, micronucleus (MN) formation has been used as a biomarker of genotoxicity, and replicative and mitotic indices (MIs) as biomarkers of cell cycle kinetics in human lymphocytes. Cells were cultured either as whole blood or isolated lymphocytes and treated with pure or commercial forms of 2,4-D at doses between 0.001 and 1 mM for 48 h. Exposure to 2,4-D produced a minimal increase in MN in whole blood and even smaller one in isolated lymphocyte cultures. This induction took place only at levels approaching cytotoxicity and was accompanied by a significant inhibition of replicative index (RI). At a low (0.005 mM) dose of commercial 2,4-D, a small, marginally significant increase in RI (12-15%) was found in two independent sets of experiments ($P = 0.052$). Additionally, we found that lymphocyte RI was more affected by commercial 2,4-D containing 9.4% of the chemically pure 2,4-D, than with an equal concentration of the latter suggesting that other ingredients present in the commercial pesticide may be responsible or may enhance the effect of 2,4-D. Mitotic index, however, did not show any significant change with either commercial or pure 2,4-D. The lymphocytes of 12 male applicators exposed solely to 2,4-D during a 3-month period had a significantly higher RI than the same group prior to exposure and than a control group ($P < 0.01$), in accordance with the in vitro finding of increased RI at low doses. (C) 2002 Elsevier Science B.V. All rights reserved. [References: 61] Number of References 61**[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

Kornuta N, Bagley E, Nedopitanskaya N. 1996. Genotoxic effects of pesticides. J Environ Pathol Toxicol Oncol 15: 75-8.
Abstract: Epidemiologic data showed an increase in the number of cancer cases in persons involved in agricultural production using pesticides. According to IARC, more than 25% of pesticides are classified as oncogens. In recent years, the concept of malignant tumors developing after environmental contamination with chemicals has been accepted. Changes in genetic material are at the basis of this process because many environmental pollutants are chemical carcinogens and mutagens with the capacity of causing DNA damage. DNA damage was proposed as a useful parameter for assessing the genotoxic properties of environmental pollutants. The correlation between exposure to carcinogenic substance and the level of DNA damage is essential. Pesticides are highly biologically active chemicals. They may interact with DNA and damage its structure. Such interaction may be critical for the manifestation of carcinogenic properties of different chemicals. We report on the organotropic genotoxic effects of different chemical classes of pesticides (decis, cypermetrin, 2,4-D, polyram) studied by means of alkaline unwinding assay DNA.

Tuschl H, Schwab C. 2003 Mar. Cytotoxic effects of the herbicide 2,4-dichlorophenoxyacetic acid in hepg2 cells. Food & Chemical Toxicology 41:385-393.
Abstract: 2,4-Dichlorophenoxyacetic acid (2,4-D) and its derivatives are herbicides widely used to control the growth of broadleaf and woody plants. Although 2,4-D is well known to be moderately toxic, little information is available on the mechanisms of its toxicity. Results on carcinogenicity, genotoxicity and mutagenicity are contradictory, but neurotoxic, immunosuppressive and hepatotoxic effects have been defined. The aim of the present study was to investigate the cytotoxic effects of 2,4-D on a human hepatoma cell line. HepG2 cells were treated with different concentrations of 2,4-D, and cell viability, induction of apoptosis/necrosis and cell cycle phases were determined. Apoptosis was detected in flow cytometric light scatter histograms, the annexin V assay, the determination of DNA strand breaks with the TUNEL assay and the occurrence of a sub G(0) peak after propidium iodide (PI) staining. The induction of apoptosis by 2,4-D was accompanied by a disruption of the mitochondrial membrane potential as verified by staining with the cationic JG-1 probe. In addition, 2,4-D affected the cell cycle in a concentration-dependent manner. Our investigation suggested that 2,4-D exerts its cytotoxic effects by the induction of apoptosis via a direct effect on the mitochondrial membrane potential. (C) 2002 Elsevier Science Ltd. All rights reserved. [References: 26] Number of References 26

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. [References: 40] Number of References 40

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:

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: 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.

Sister chromatid exchanges and some other mutagenic effects of 2,4-D or its formulations at various doses were also caused in the following experiments:

E. Madrigal-Bujadar et al. 2001 Sep. 'Induction of sister chromatid exchanges by 2,4-dichlorophenoxyacetic acid in somatic and germ cells of mice exposed in vivo' Food Chem Toxicol.:39(9):941-6.

Turkula TE & Jalal SM.1985 May-Jun 'Increased rates of sister chromatid exchanges induced by the herbicide 2,4-D' J Hered.:76(3):213-4.

Korte C & Jalal SM. . 1982 May-Jun '2,4-D induced clastogenicity and elevated rates of sister chromatid exchanges in cultured human lymphocytes' J Hered:73(3):224-6.

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]

Jenssen D, Renberg L. 1976 Aug. Distribution and cytogenetic test of 2,4-D and 2,4,5-T phenoxyacetic acids in mouse blood tissues. Chem Biol Interact 14:279-89.

Abstract: The phenoxyacetic acids 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), extensively used as herbicides, were tested for cytogenetic effects by means of induced micronuclei in erythrocytes of mouse bone marrow. Because of the hig experimental resolution power this is a particularly suitable test system for the detection of weak chromosome breaking activity in mammals. The cytogenetic tests were supplemented with chemical analyses of the concentration the the test substances reaching the target cells...

Schop RN, Hardy MH, Goldberg MT. 1990 Nov. Comparison of the activity of topically applied pesticides and the herbicide 2,4-D in two short-term in vivo assays of genotoxicity in the mouse. Fundam Appl Toxicol 15:666-75.
Abstract: Genotoxicity of eight topically applied compounds was determined using the bone marrow micronucleus (MN) test and hair follicle nuclear aberration (NA) assay in CD1 mice. Twenty-four hours after a single treatment, cyclophosphamide (CY), applied at doses corresponding to 1/4, 1/8, 1/16, and 1/32 of the published dermal LD50, and N-methyl-N-nitrosourea (MNU), applied at 1/4, 1/8, and 1/16 of the published dermal LD50, were found to increase the incidence of NA in a dose-dependent manner. The frequency of MN was significantly increased only at the highest dose of CY. Using the same protocol, six pesticides applied in dimethyl sulfoxide (DMSO) at doses of 1/8, 1/16, and 1/32 of the dermal LD50 were investigated. Aminocarb and chlordane induced a dose-dependent increase in the frequency of NA, while there was an observed increase in NA incidence at only the highest doses of dichlorvos (DDVP), 4,4'-DDT (DDT), and 2,4-dichlorophenoxyacetic acid (2,4-D). No effect was observed with fenitrothion on nuclear aberrations in hair follicles. Except for the highest dose of chlordane, none of the pesticides tested positive in the bone marrow micronucleus test. Serum cholinesterase levels were reduced to 70 +/- 4.7% of the DMSO control level with DDVP, 57 +/- 8.2% with aminocarb, and 60.3 +/- 4.8% with fenitrothion, indicating some systemic activity with these topically applied agents. The data suggest that aminocarb, chlordane, DDVP, DDT, and 2,4-D are genotoxic as determined by the NA assay and that this assay may be more useful in detecting topically applied genotoxic agents than the more often used bone marrow micronucleus test.

Mustonen R, Kangas J, Vuojolahti P, Linnainmaa K. 1986 Jul. Effects of phenoxyacetic acids on the induction of chromosome aberrations in vitro and in vivo. Mutagenesis 1:241-5.
Abstract: The effects of phenoxyacetic acid herbicides were investigated on the induction of chromosome aberrations in human peripheral lymphocyte cultures in vitro and in lymphocytes of exposed workers in vivo. Pure 2,4-dichlorophenoxyacetic acid (2,4-D; 0.125, 0.150, 0.200 and 0.350 mM) did not increase the number of aberrations, whereas the commercial 2,4-D formulation (0.125, 0.250, 0.500, 1.000 and 1.250 mM, with respect to phenoxyacetic acid concentration) significantly increased the number of chromosome aberrations in vitro (without exogenous metabolic activation). The phenoxy acid levels in the breathing zone of the workers varied between 0.3 and 0.4 mg/m3, and the concentrations of phenoxyacetic acids in the urine of the workers after exposure varied from 0.000 to 0.055 mmol/l. There were no increases in chromosome aberrations in peripheral lymphocytes of the exposed subjects. ---

Riabchenko NI, Fesenko EV, Antoshchina MM. 1995 Sep-Oct. [A cytogenetic analysis of the combined action of pesticides and irradiation on human lymphocytes]. Radiats Biol Radioecol 35:736-9.

Abstract: The efficiency of the combined action of pesticides and irradiation at the G(o) stage was studied in cultured human lymphocytes. Carbophos (malathion) increased the yield of chromosome and chromatid fragments in irradiated lymphocytes. Herbicide 2,4-D (dichlorophenoxyacetic acid) raised lymphocyte radiosensitivity by increasing the yield of chromosome type aberrations; the radiosensitizing effect of the herbicide decreased as its concentration increased. ---

Venkov P, Topashka-Ancheva M, Georgieva M, Alexieva V, Karanov E. 2000 Nov. Genotoxic effect of substituted phenoxyacetic acids. Arch Toxicol 74:560-566.

Abstract: The potential toxic and mutagenic action of 2,4-dichlorophenoxyacetic acid has been studied in different test systems, and the obtained results range from increased. chromosomal damage to no effect at all. We reexamined the effect of this herbicide by simultaneous using three tests based on yeast, transformed hematopoietic, and mouse bone marrow cells. The results obtained demonstrated that 2,4-dichlorophenoxyacetic acid: has cytotoxic and mutagenic effects. The positive response of yeast and transformed hematopoietic cells was verified in kinetics and dose-response experiments. The analysis of metaphase chromosomes indicated a statistically proved induction of breaks, deletions, and exchanges after the intraperitoneal administration of 2,4-dichlorophenoxyacetic acid in mice. The study of phenoxyacetic acid and its differently chlorinated derivatives showed that cytotoxicity and mutagenicity are induced by chlorine atoms at position 2 and/or 4 in the benzene ring. The mutagenic effect was abolished by introduction of a third chlorine atom at position 5. Thus 2,4,5-trichlorophenoxyacetic acid was found to have very weak, if any mutagenic effect; however, the herbicide preserved its toxic effect. [References: 25] Number of References 25 Keywords: ---

Zeljezic D, Garaj-Vrhovac V. 2004 Jul 15. Chromosomal aberrations, micronuclei and nuclear buds induced in human lymphocytes by 2,4-dichlorophenoxyacetic acid pesticide formulation. Toxicology 200:39-47.

Abstract: Pesticides of worldwide application are used in agriculture in vast amounts each year, of which herbicides are the most prominent class. Phenoxyacetic herbicides constitute one of the largest groups of herbicides sold in the world. Among them, for many years 2,4-dichlorophenoxyacetic acid (2,4-D) has been the one most used. In this study we used Deherban A, a commercial formulation of 2,4-D to determine its possible genotoxic effect on human lymphocytes in vitro by chromosomal aberration analysis and micronucleus assay including the scoring of nuclear buds. Two different concentrations of pesticide formulation were used so that final concentrations of 2,4-D were 0.4 and 4 microg/ml, both in the presence and in the absence of the liver microsomal fraction as metabolic activator. Both concentrations of pesticide caused an increase in chromatid and chromosome breaks, number of micronuclei and number of nuclear buds. Presence of the S9 mix additionally elevated the number of chromatid breaks and micronuclei in treated lymphocytes.

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.

Tuschl H, Schwab CE. 2004 Aug. Flow cytometric methods used as screening tests for basal toxicity of chemicals. Toxicology in Vitro 18:483-491. **Abstract:** Aim of the present study was to evaluate the suitability of flow cytometry to test in vitro effects of toxicants. Flow cytometry offers the possibility to study several parameters simultaneously, e.g. cell cycle modulation, apoptosis and necrosis within the same cell culture. The effects of six compounds (acetaminophen = AAP, isoniazid = INH, digoxin, malathion, paraquat and 2,4-dichlorophenoxy acetic acid = 2,4-D) on cell cycle were investigated in HepG2 cells and the induction of apoptosis/necrosis was analyzed by a spectrum of flow cytometric assays in HepG2, AAH-1 and YAC-1 cells. Early indicators of apoptosis-loss of mitochondrial membrane polarization-as well as later events of the apoptotic process-annexin V binding and DNA fragmentation-were studied. The phases of the cell cycle and the occurrence of a sub-Go peak of apoptotic cells were determined with propidium iodide staining. The present investigation demonstrated good correlations between results obtained by flow cytometric analyses and the IC50 data of the MEIC (= Multicenter Evaluation of In Vitro Cytotoxicity) study. Regarding the short time required for the tests, the possibility of investigating several parameters of cytotoxicity simultaneously and the ease of performance, flow cytometric analyses are well suited for the pre-screening for toxic effects of chemicals. (C) 2004 Elsevier Ltd. All rights reserved. [References: 22] Number of References 22

Linnainmaa K. 1984. The effects of hypolipidemic peroxisome proliferators on the induction of sister chromatid exchanges. Basic Life Sci 29 Pt B:965-74.

AMBIGOUS RESULT: MUTAGENIC

Linnainmaa K. 1984 Jun. Induction of sister chromatid exchanges by the peroxisome proliferators 2,4-D, MCPA, and clofibrate in vivo and in vitro. Carcinogenesis 5:703-7.
Abstract: Phenoxy acid herbicides 2,4-dichlorophenoxyacetic acid (2,4-D) and 4-chloro-2-methylphenoxyacetic acid (MCPA) have been found to induce proliferation of peroxisomes in the liver cells of rodents in the same manner as the hypolipidemic drug clofibrate. Both phenoxy acid herbicides and clofibrate (ethyl-alpha-p- chlorophenoxyisobutyrate) are suspected carcinogens. The present study reports the effect of these agents on the induction of sister chromatid exchange (SCE) in the blood lymphocytes of exposed rats (100 mg/kg with 2,4-D and MCPA, 200 mg/kg with clofibrate for 2 weeks in one intragastric dose/day), in the bone marrow cells of exposed Chinese hamsters (100 mg/kg, treatments as above), and in Chinese hamster ovary (CHO) cells in vitro (10(-5), 10(-4), and 10(-3) M, for 1 h). In the experiments in vitro, the effects of purified 2,4-D and MCPA phenoxy acids were studied, in addition to those of the commercial herbicide formulations and clofibrate. No increase of SCE frequency was observed in the blood lymphocytes of the exposed rats in comparison with the controls. In the bone marrow cells of the exposed Chinese hamsters, a slight increase of SCE was found in the group treated with MCPA but not in the groups treated with 2,4-D or clofibrate. A slight increase in the number of SCEs was characteristic of all the treated CHO cell cultures, both with and without a rat liver microsomal activation system (S9 mix). No clear dose-related effects, however, could be discerned with any of the compounds, and no differences in the SCE induction were observed between the commercial herbicide products and the purified phenoxy acetic acids. The present results support the data which indicate that 2,4-D, MCPA, and clofibrate do not act as direct DNA-damaging agents.

======

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

Charles JM et al. 1999 Jul 21. [title unknown] Mutation Research 444:217-226.
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

Linnainmaa K. 1983. Sister chromatid exchanges among workers occupationally exposed to phenoxy acid herbicides 2,4-D and MCPA. Teratog Carcinog Mutagen 3:269-79.
Abstract: The induction of sister chromatid exchanges (SCEs) was studied in the peripheral lymphocytes of workers spraying foliage in forestry with phenoxy acid herbicides 2,4-dichlorophenoxyacetic acid (2,4-D) and 2-methyl-4-chlorophenoxyacetic acid (MCPA) or their mixtures. In order to follow possible exposure-related changes in the frequencies of SCEs, three successive blood samples were taken from 50 male sprayers during the spraying season of July-October, 1981. In addition, 15 control subjects not working with herbicides were included in the study. The actual exposure levels of the exposed subjects were estimated by measuring the concentrations of 2,4-D and MCPA in the urine of the sprayers. Enough cells for the SCE analysis were obtained from 35 herbicide workers and 15 control subjects. The concentrations of 2,4-D and MCPA in the urine samples after exposure varied from 0.00 to 10.99 mg/l. No significant differences in the frequencies of SCEs were observed in samples taken before, during, or after the exposure. Furthermore, the means of SCEs in a nonexposed control group of 15 subjects fell in the same range as those of the exposed subjects. A difference in the means of SCEs was observed between nonsmokers and smokers, smokers having significantly higher mean values than nonsmokers. The results of the present study add support to the earlier data indicating that 2,4-D and MCPA do not act as direct DNA-damaging agents.

=====

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 of 2,4-D, because both the epidemiological and the mechanistic literature strongly support 2,4-D causing immune cancers. These results create considerable doubt about the Agency method of reliance on evaluations of animal studies used to classify 2,4-D as having insufficient evidence of carcinogenicity.

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.
OBJECTIVE: Evaluate peripheral blood lymphocyte proliferation (replicative index:RI) and micronuclei frequency (MF) among 2,4-D herbicide applicators. METHODS: Twelve applicators spraying only 2,4-D provided a blood and urine specimen upon enrollment, several urine samples during the spraying season, and a blood specimen at the study's end. Nine controls provided blood and urine specimens upon enrollment and at the study's end. Gas chromatography/tandem mass spectroscopy determined urinary 2,4-D levels and standard in-vitro assays

determined RI and MF scores. Applicator RI and MF were compared before and after spraying and with controls. RESULTS: Applicators contributed 45 urine specimens with concentrations ranging from 1.0 to 1700 (microg 2,4-D/g creatinine/L urine) that logarithmically (In) increased as spraying time increased. Applicator RI increased after spraying ($p = 0.016$), independent of tobacco and alcohol use, and demonstrated a weak dose-response with increasing urinary 2,4-D levels ($p = 0.15$). Among 2,4-D applicators, pre-exposure complete blood counts and lymphocyte immunophenotypes were not significantly different from post-exposure measurements. CONCLUSION: Urinary 2,4-D concentration, an exposure biomarker, may be associated with lymphocyte replicative index, a cell proliferation biomarker.
[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. [References: 61] Number of References 61[ALSO IN 'MUTAGENIC/POSITIVE' RESULTS. **NOTE THE SUPER-LOW (5 PICOMOLE) DOSE EFFECT**]

Kaioumova D, Susal C, Opelz G. 2001 Jan. Induction of apoptosis in human lymphocytes by the herbicide 2,4-dichlorophenoxyacetic acid. Hum Immunol 62:64-74.

Abstract: Dimethylammonium salt of 2,4-dichlorophenoxyacetic acid (DMA-2,4-D) is a widely used herbicide that is considered moderately toxic. In the present study we found that DMA-2,4-D is able to cause apoptosis in peripheral blood lymphocytes of healthy individuals and Jurkat T cells. Apoptosis induced by DMA-2,4-D was dose and time dependent, independent of Fas, TNF receptor 1 or the aromatic hydrocarbon receptor, and involved disruption of the mitochondrial transmembrane potential and activation of caspase-9. ZVAD-FMK, a broad-spectrum inhibitor of caspases, blocked DMA-2,4-D-induced apoptosis completely. While an inhibitor of caspase-9, as well as caspase-9 and caspase-3 inhibitors in combination, strongly blocked DMA-2,4-D-induced apoptosis, an inhibitor of caspase-3 had a moderate inhibitory effect. Unlike Fas-mediated apoptosis, the initiator caspase, caspase-8, was not involved in DMA-2,4-D-induced apoptosis. Transfection of Jurkat cells with Bcl-2 prevented DMA-2,4-D-induced disruption of the mitochondrial transmembrane potential and led to a complete blockage of apoptosis. Our data indicate that DMA-2,4-D kills human lymphocytes by initiating apoptosis via a direct effect on mitochondria. The activation of caspases occurs downstream of mitochondrial damage, and the dysfunction of mitochondria appears to be sufficient for triggering all downstream events leading to apoptosis.

Lin N, Garry VF. 2000. *In vitro* studies of cellular and molecular developmental toxicity of adjuvants, herbicides, and fungicides commonly used in Red River Valley, Minnesota. Journal of Toxicology & Environmental Health - Part A 60:423-439.

Abstract: Recent epidemiologic studies showed increased frequency of birth defects in pesticide applicators and general population of the Red River Valley, Minnesota. These studies further indicated that this crop growing area used more chlorophenoxy herbicides and fungicides than elsewhere in Minnesota. Based on frequency of use and known biology, certain herbicides, pesticide additives, fungicides, and mycotoxins and suspect agents. To define whether these agents affect developmental endpoints *in vitro*, 16 selected agrochemicals were examined using the MCF-7 breast cancer cell line. In the flow cytometric assay, cell proliferation in this estrogen-responsive cell line indicates xenobiotic-mediated estrogenic effects. Cell viability, morphology, ploidy, and apoptosis were incorporated in this assay. Data showed that the adjuvants X-77 and Activate Plus induced significant cell proliferation at 0.1 and 1 ìg/ml. The commerical-grade herbicide 2,4-D LV4 and 2,4-D amine induced cell proliferaton at 1 and 10 ìg/ml. The reagent-grade 2,4-D products failed to induce proliferation over the same concentration range, suggesting that other ingredients in the commercial products, presumably adjuvants, could be a factor in these results. The fungicides triphenyltin and mancozeb induced apoptosis at concentration of 4.1 ìg/ml (10-5 M). Data provide a mechanistic step to understanding human reproductive and developmental effects in populations exposed to these agrochemicals, and initiative to focusing limited resources for future use *in vivo* animal developmental toxicity studies.

Merillon JM, Filali M, Duperon P, Montagu M, Chenieux JC, Rideau M. 1995 Jul-1995 Aug 31. Effect of 2,4-dichlorophenoxyacetic acid and habituation on lipid and protein composition of microsomal membranes from periwinkle cell suspensions. Plant Physiology & Biochemistry 33:443-451.
Abstract: Investigations on biochemical changes associated with habituation in plant cells and tissues grown in vitro are still Limited. In the present work, we have compared the lipid and protein composition of microsomal membranes prepared from two Catharanthus roseus cell lines: a 2,4-dichlorophenoxyacetic acid (2,4-D)-dependent line and a fully-habituated line selected from the former. In order to distinguish changes associated with habituation from those associated with 2,4-D treatment, each line was grown for one passage in medium with or without 2,4-D. The auxin Provoked a lower amount of phospholipid, a higher free-sterol to phospholipid ratio, and a decreased fluidity in microsomal membranes, all parameters usually associated with cell senescence. On the other hand, habituation decreased the free sterol to phospholipid ratio, increased the oleic add to linoleic acid ratio and the sitosterol to campesterol ratio. The fluidity of the membranes from habituated cells increased. Habituation, as well as treatment of the cells with 2,4-D, changed the polypeptide profiles of the microsomal membranes. The data lead to the conclusion that membranes are targets for biochemical changes associated with habituation. They also support the hypothesis that some similarities exist between habituated cells and animal tumour cells. [References: 38] Number of References 38

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

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

Faustini A, Settimi L, Pacifici R, Fano V, Zuccaro P, Forastiere F. 1996. Immunological changes among farmers exposed to phenoxy herbicides - Preliminary observations. Occupational & Environmental Medicine 53:583-585.
Abstract: Objectives-To evaluate short term immunological changes after agricultural exposure to commercial formulations of chlorophenoxy herbicides. Methods-Blood samples were collected hom 10 farmers within seven days before exposure, one to 12 days after exposure, and again 50 to 70 days after exposure. Whole blood was used to count lymphocyte subsets with monoclonal antibodies. Peripheral blood mononuclear (PBM) cells were used to measure natural killer (NK) cell activity and lymphocyte response to mitogenic stimulations. Values

before exposure were used as reference. Results-in comparison with concentrations before exposure, a significant reduction was found one to 12 days after exposure in the following variables (P <0.05): circulating helper (CD4) and suppressor T cells (CD8), CD8 dim, cytotoxic T lymphocytes (CTL), natural killer cells (NK), and CD8 cells expressing the surface antigens HLA-DR (CD8-DR), and lymphoproliferative response to mitogen stimulations. All immunological values found 50-70 days after exposure were comparable with concentrations before exposure, but mitogenic proliferative responses of lymphocytes were still significantly decreased. Conclusions-According to our data agricultural exposure to commercial 2,4-dichlorophenoxyacetic acid (2,4-D) and 4-chloro-2-methylphenoxyacetic acid (MCPA) formulations may exert short term immunosuppressive effects. Further studies should clarify whether the immunological changes found may have health implications and can specifically contribute to cancer aetiology.[IMMUNE PARAMETERS INCREASED AFTER EXPOSURE, THEN DECREASED--A HINT OF CAUSATION:]

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 and 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 B63F1 mice, 27 days of age, were exposed to 2,4-D by gavage at doses of 0, 3, 10, 30, and 100 mg/ kg/day for 4 successive days and sacrificed on day 5. Thymus DNA was isolated and examined for illegitimate V(D)J recombination-mediated gene rearrangements. In addition, pregnant mice were exposed to 2,4-D and thymocytes from the offspring examined at 2 weeks of age. No significant increase in aberrant V(D)J rearrangements was found, indicating that under these conditions 2,4-D does not appear to effect this important mechanism of carcinogenesis. [References: 40] Number of References 40 =====

MECHANISM/PEROXISOME PROLIFERATION

2,4-D is an undisputed peroxisome proliferater (PP). But PP's role in cancer in different species is the subject of a legitimate and vigorous scientific debate. Yet it is *not disputed* that PPs, and thus 2,4-D, are **risk factors** for cancer in various vertebrate and other animal species, if not in humans.

POSITVE RESULTS: MECHANISM/PEROX. PROLIF.

Ge R, Tao L, Kramer PM, Cunningham ML, Pereira MA. 2002. Effect of peroxisome proliferators on the methylation and protein level of the c-myc protooncogene in B6C3F1 mice liver. J Biochem Mol Toxicol 16:41-7. **Abstract:** Peroxisome proliferators in general are nongenotoxic mouse liver carcinogens for which DNA hypomethylation and altered gene expression are proposed mechanisms. Therefore, the peroxisome proliferators 2,4-dichlorophenoxyacetic acid (2,4-D), dibutyl phthalate (DBP), gemfibrozil, and Wy-14,643 were evaluated for the ability to alter the methylation and expression of the c-myc protooncogene. Male B6C3F1 mice were administered for 6 days in their diet Wy-14,643 (5-500 ppm), 2,4-D (1,680 ppm), DBP (20,000 ppm), or gemfibrozil (8,000 ppm). All four peroxisome proliferators caused hypomethylation of the c-myc gene in the liver. Wy-14,643 appeared to be the most efficacious with a threshold between 10 and 50 ppm. The level of the c-myc protein was increased by Wy-14,643, but not the other peroxisome proliferators. When female B6C3F1 mice received a two-thirds partially hepatectomy and 16 h later were administered 50 mg/kg Wy-14,643 by gavage, hypomethylation of the gene occurred 24 h later. Hypomethylation was not found in mice that received

Wy-14,643 following a sham operation. Hypomethylation of the c-myc gene within 24 h of administering Wy-14,643 after a partial hepatectomy but not after a sham operation supports the hypothesis that the peroxisome proliferators prevent methylation of hemimethylated sites formed by DNA replication.

Vainio H, Nickels J, Linnainmaa K. 1982 Mar. Phenoxy acid herbicides cause peroxisome proliferation in Chinese hamsters. Scand J Work Environ Health 8:70-3.

Abstract: An increase in either the size or amount of peroxisomes was obtained in the liver cells of Chinese hamsters after the animals were exposed to the phenoxy herbicides 2,4-dichlorophenoxyacetic acid (2,4-D) or 4-chloro-2-methylphenoxyacetic acid (MCPA). At the dose level studied, 2,4-D was found to be more potent than MCPA in increasing the number of peroxisomes. A phenoxy acid derivative, clofibrate, one of the peroxisome proliferators known to possess carcinogenic properties in rodents, appeared to be still more potent in inducing peroxisome proliferation than either of the herbicides studied. Further investigations are warranted to clarify the significance of peroxisome proliferation to the toxicity of phenoxy herbicides.

Biochem Pharmacol. 1983 Sep 15;32(18):2775-9. Hypolipidemia and peroxisome proliferation induced by phenoxyacetic acid herbicides in rats. Vainio H, Linnainmaa K, Kahonen M, Nickels J, Hietanen E, Marniemi J, Peltonen P.

Male Wistar rats were treated daily by gavage with two phenoxy herbicides, 2,4-dichlorophenoxyacetic acid (2,4-D)(100-200 mg/kg body wt) and 4-chloro-2-methylphenoxyacetic acid (MCPA) (100-200 mg/kg body wt), and with the chemically different glyphosate N-phosphonomethyl glycine (300 mg/kg body wt) 5 days per week for 2 weeks. A hypolipidemic drug, clofibrate [ethyl-2-(4-chlorophenoxy)-2-methylpropionate], which is structurally related to phenoxy acids, was used as a positive control (200 mg/kg body wt). 2,4-D and MCPA had several effects similar to those of clofibrate: all three compounds induced proliferation of hepatic peroxisomes, decreased serum lipid levels, and increased hepatic carnitine acetyltransferase and catalase activities. 2,4-D and MCPA, but not clofibrate, decreased lipoprotein lipase activity in the adipose tissue to about a third of the control value but did not change the lipoprotein lipase activity in the heart muscle. The data suggest that these compounds cause hypolipidemia not by enhancing the storage of peripheral lipids in adipose tissue but by preferentially increasing lipid utilization in the liver. Glyphosate caused no peroxisome proliferation or hypolipidemia, suggesting that these effects are associated with the structural similarity between phenoxy acid herbicides and clofibrate.

PMID: 6626247 [PubMed - indexed for MEDLINE]

Acta Pharmacol Toxicol (Copenh). 1983 Aug;53(2):103-12. Effects of phenoxyherbicides and glyphosate on the hepatic and intestinal biotransformation activities in the rat. Hietanen E, Linnainmaa K, Vainio H.

The effects of phenoxyacid herbicides 2,4-D (2,4-dichlorophenoxyacetic acid) and MCPA (4-chloro-2-methylphenoxyacetic acid), clofibrate, and glyphosate on hepatic and intestinal drug metabolizing enzyme activities were studied in rats

intragastrically exposed for 2 weeks. The hepatic ethoxycoumarin O-deethylase activity increased about 2-fold with MCPA. Both 2,4-D and MCPA increased the hepatic epoxide hydrolase activity and decreased the hepatic glutathione S-transferase activity. MCPA also increased the intestinal activities of ethoxycoumarin O-deethylase and epoxide hydrolase. Glyphosate decreased the hepatic level of cytochrome P-450 and monooxygenase activities and the intestinal activity of aryl hydrocarbon hydroxylase. Clofibrate decreased the hepatic activities of UDPglucuronosyltransferase with p-nitrophenol or methylumbelliferone as the substrate. Also 2,4-D decreased the hepatic activity of UDPglucuronosyltransferase with p-nitrophenol as the substrate. MCPA decreased the intestinal activities of UDPglucuronosyltransferase with either p-nitrophenol or methylumbelliferone as the substrate. The results indicate that phenoxyacetic acids, especially MCPA, may have potent effects on the metabolism of xenobiotics. Glyphosate, not chemically related to phenoxyacids, seems to inhibit monooxygenases. Whether these changes are related to the toxicity of these xenobiotics remains to be clarified in further experiments. PMID: 6624478 [PubMed - indexed for MEDLINE]

======

NEGATIVE RESULTS: MECHANISM/PEROX. PROLIF.

Abdellatif AG, Preat V, Vamecq J, Nilsson R, Roberfroid M. 1990 Nov. Peroxisome proliferation and modulation of rat liver carcinogenesis by 2,4-dichlorophenoxyacetic acid, 2,4,5-trichlorophenoxyacetic acid, perfluorooctanoic acid and nafenopin. Carcinogenesis 11:1899-902.
Abstract: Using an initiation--selection--promotion protocol for induction of liver tumors in Wistar rats, the modulating action of various peroxisome proliferators on neoplasia as well as on selected biochemical parameters was studied. After treatment with diethylnitrosamine (DEN), the animals were subsequently subjected to a selection procedure involving feeding of 2-acetylaminofluorene (2-AAF), and in the middle of the 2-AAF treatment, a single necrogenic dose of carbon tetrachloride. Following a recovery period, the rats were fed a diet containing 0.1% nafenopin (NAF), 0.015% perfluorooctanoic acid (PFOA), 0.05% 2,4-dichlorophenoxyacetic acid (2,4-D), 0.05% 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) or 0.05% phenobarbital (PB) as a positive control. When the animals were killed, 7 months after initiation, the incidence of hepatocellular carcinoma was 83, 33 and 16% in the animals treated with NAF, PFOA or 2,4,5-T respectively. No cancers were observed in controls, or in the 2,4,-D groups. In comparison with controls, NAF and PFOA caused a 60-and 24-fold increase inthe peroxisomal beta-oxidation of fatty acids respectively, but only about a 2-fold increase in the catalase activity, 2,4-D and/or 2,4,5-T were much less active in this respect, giving approximately a doubling in the rate of fatty acid oxidation. The specific activity of D-amino acid and glycolate oxidases were significantly depressed, whereas the urate oxidase levels were apparently unaffected by the NAF and PFOA treatment. The results suggest that the selective

induction of peroxisomal fatty acid oxidation is consistent with the hypothesis that imbalance between H2O2 overproduction and its destruction could play a role in the modulation of hepatocarcinogenesis by peroxisome proliferators.

Mikalsen SO, Ruyter B, Sanner T. 1990 Feb 1. Effects of hepatic peroxisome proliferators and 12-O-tetradecanoyl phorbol-13-acetate on catalase and other enzyme activities of embryonic cells in vitro. Biochem Pharmacol 39:527-35.
Abstract: The effects of the hepatic peroxisome proliferators (HPPs) clofibrate, di-(2-ethylhexyl)-phthalate (DEHP), mono-(2-ethylhexyl)phthalate (MEHP) and 2,4-dichlorophenoxy acetic acid (2,4-D) on the activities of some peroxisome-associated enzymes and marker enzymes for other organelles, have been studied in primary Syrian hamster embryo (SHE) cells and Wistar rat embryo (WRE) cells. The majority of the cells are fibroblast-like. 12-O-Tetradecanoyl phorbol-13-acetate (TPA) was included as it has been suggested that it may act as a peroxisome proliferator. The specific activities of catalase, fatty acyl-CoA oxidase (FAO) and peroxisomal beta-oxidation were approximately 100-fold lower in the embryonic cells than in rat hepatocytes. Other peroxisome-associated oxidases were not detected. The dihydroxyacetone-phosphate acyltransferase (DHAPAT) activity was comparable to that in rat liver. Marker enzymes for other organelles had specific activities comparable to rat hepatocytes. Catalase was shown by digitonin titration to be contained in a peroxisome-like compartment in both SHE and WRE cells. Clofibrate, DEHP and MEHP increased the catalase activity, which might suggest peroxisome proliferation. However, the findings that FAO and peroxisomal beta-oxidation did not increase or only very slightly, argue against peroxisome proliferation. 2,4-D and TPA induced no or only a very slight increase in the catalase activity.

# # #