NRDC
THE EARTH'S BEST DEFENSE

NATURAL RESOURCES DEFENSE COUNCIL'S PETITION TO REVOKE ALL TOLERANCES AND CANCEL ALL REGISTRATIONS FOR THE PESTICIDE 2,4 D

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The Natural Resources Defense Council (NRDC) petitions EPA to revoke all tolerances and cancel all registrations for the pesticide 2,4-dichlorophenoxyacetic acid (2,4-D). This petition is filed pursuant to 21 U.S.C. § 346a(d) and the Administrative Procedure Act, 5 U.S.C. § 551 et seq.

INTRODUCTION

2,4-D (2,4-dichlorophenoxyacetic acid) is a common herbicide that has been registered in the United States since 1948 and is therefore one of the oldest pesticides still legally on the market. About 46 million pounds of 2,4-D are used in the U.S. annually, making it the third most widely used herbicide in North America.[1] Two-thirds of the use of 2,4-D is in agriculture including on pasture land, wheat, corn, soybeans, barley, rice, oats, and sugar cane. The other third of its use is in a variety of non-agricultural settings, including direct sale to homeowners for lawn and garden use, to control aquatic weeds in water where people may swim, and on, for example, athletic fields, golf courses, and playgrounds. In fact, it is the most commonly used conventional pesticide active ingredient in the "home and garden" market, as well as the industry/commercial/government market.[2] Although more effective and less toxic alternatives are available, 2,4-D remains popular because of its low cost.

2,4-D has a soil half-life of about one week; however, 2,4-D is found as a contaminant in about half of all surface water samples across the U.S. and has been detected in groundwater in at least five states and Canada.[3] Also, when tracked indoors and not

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[1] Environmental Protection Agency, Reregistration Eligibility Decision for 2,4-D, June 2005, xi.
[2] 2,4-D. HED's Human Health Risk Assessment for the Reregistration Eligibility Decision (RED) Revised to Reflect Error-only Comments from Registrants. 2 June 2004. 62.
[3] Extension Toxicology Network. 1993. http://extoxnet.orst.edu/pips/24-D.htm (citing Howard, Philip H. Handbook of Environmental Fate and Exposure Data for Organic Chemicals. Lewis Publishers Chelsea, Michigan.)

exposed to direct sunlight, 2,4-D persists in carpets for up to one year after a single turf application at a concentration of approximately 0.5μg/g.[4]

2,4-D is a phenoxy herbicide. Other herbicides in this class include several that are still registered for use, including: 2-methyl-4-chlorophenoxyacetic acid (MCPA), 2-(2-methyl-4-chlorophenoxy)propionic acids (mecoprop, MCPP), 2-(2,4-dichlorophenoxy)propionic acid (dichloroprop, 2,4-DP), (2,4-dichlorophenoxy)butyric acid (2,4-DB). Another chemical in the same class, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), was combined with 2,4-D during the Vietnam War to make Agent Orange. Due to the significant health and environmental concerns associated with Agent Orange, 2,4,5-T use was terminated by EPA in 1985.

EPA published a Reregistration Eligibility Decision (RED) for 2,4-D on August 8, 2005. The RED was largely based on Revised Risk Assessments and Preliminary Risk Reduction Options published on January 12, 2005. In the RED, EPA concluded that 2,4-D was eligible for reregistration, with only minor use and labeling changes. Furthermore, the Agency concluded that the tolerances for 2,4-D met the Food Quality Protection Act (FQPA) safety standards for the U.S. population and sensitive populations, including infants and children. EPA found that there was a reasonable certainty of no harm to the general population and any subgroup from the use of 2,4-D.[5]

As discussed in this petition, the state of the science identifying many various adverse health effects associated with exposure to 2,4-D requires that 2,4-D be banned and all food tolerances revoked.

This petition also highlights the weaknesses in the RED which undermine EPA's conclusions that 2,4-D should be re-registered and its food tolerances retained. The deficiencies in the risk assessments relate to the toxicity of 2,4-D and the amount of human exposure to the chemical. First, EPA failed to incorporate information on the endocrine disrupting effects of 2,4-D into its ecological or human health risk assessments, and improperly ignored data showing adverse effects in aquatic species when it approved 2,4-D for use in or near water. Second, EPA disregarded data on neurotoxicity related to 2,4-D exposure. Third, EPA disregarded information showing that widely marketed pesticide formulations containing 2,4-D are mutagenic. Fourth, EPA ignored data showing that dermal absorption of 2,4-D is enhanced by alcohol consumption, sunscreen and DEET, failed to incorporate that information into the exposure assessment, and failed to adopt any risk reduction measures to prevent hazardous exposures that could result. And finally, in the aggregate risk assessment EPA completely ignored the exposure of infants to 2,4-D via breast milk, and the evidence that such exposure may result in adverse developmental effects at doses below those that formed the basis for EPA's risk assessment. In light of the gaping inadequacies in the risk assessments, and in light of the

[4] 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.

[5] Environmental Protection Agency, Reregistration Eligibility Decision for 2,4-D, June 2005, 84-97.

evidence showing the dangerous effects of 2,4-D, EPA should cancel all registrations and revoke all food tolerances for 2,4-D.

LEGAL STANDARD

EPA regulates pesticides under the Federal Food, Drug, and Cosmetic Act (FFDCA), 21 U.S.C. § 346a and the Federal Fungicide, Insecticide, and Rodenticide Act (FIFRA), 7 U.S.C. § 136 *et seq.* FIFRA requires that pesticides must be registered to be sold in the United States.[6] EPA may not register a pesticide unless the chemical will perform its intended function without causing any "unreasonable adverse effects on the environment."[7]

The FFDCA authorizes EPA to set tolerances (maximum allowable levels) for pesticide residues in food or to grant exemptions from the requirement to have a tolerance.[8] EPA may "establish or leave in effect a tolerance for a pesticide chemical residue in or on a food only if the Administrator determines that the tolerance is safe."[9] The term "safe" means that "there is a reasonable certainty that no harm will result from aggregate exposure" to the pesticide, "including all anticipated dietary exposures and all other exposures for which there is reliable information."[10] A pesticide may not be used on a particular food unless there is a tolerance or exemption for that food.[11]

The FFDCA, as amended by the FQPA, explicitly requires that, in establishing a tolerance, EPA must assess the risk that a pesticide poses to infants and children in particular.[12] Before EPA can establish a tolerance, the Agency shall "ensure that there is a reasonable certainty that no harm will result to infants and children from aggregate exposure" to the pesticide and shall "publish a specific determination regarding the safety of the pesticide chemical residue for infants and children."[13] In ensuring that the statutory safety standard is met, EPA must consider available information concerning "the special susceptibility of infants and children," including "neurological differences between infants and children and adults, and effects of *in utero* exposure to pesticide chemicals."[14] EPA must also base its tolerance decision on available information about "food consumption patterns unique to infants and children" and the "cumulative effects on infants and children of [pesticides] that have a common mechanism of toxicity."[15] EPA concedes that, when setting new tolerances under the standard, the Agency "must now focus explicitly on exposures and risks to children and infants."[16]

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[6] 7 U.S.C. § 136a.
[7] 7 U.S.C. § 136a(c)(5)(C).
[8] 21 U.S.C. §§ 345a(b) & (c).
[9] § 346a(b)(2)(A)(i).
[10] § 346a(b)(2)(A)(ii).
[11] § 346a(a)(1).
[12] § 346a(b)(2)(C).
[13] §§ 346a(b)(2)(C)(ii)(I) & (II).
[14] § 346a(b)(2)(C)(i)(II).
[15] §§ 346a(b)(2)(C)(i)(I) & (III).
[16] See EPA, Fact Sheet: Protecting Children from Pesticides (Jan. 2002) (www.epa.gov/pesticides/factsheets/kidpesticide.htm) ("The 1996 Food Quality Protection Act set tougher standards to protect infants and children from pesticide risks.").

ENDOCRINE DISRUPTING EFFECTS

2,4-D has been shown to have extensive hormone-disrupting activity, including anti-thyroid, androgenic, and estrogenic effects, in addition to effects on progesterone and prolactin. These effects may occur at low doses and should be included in the risk assessment for 2,4-D. Oddly, EPA reregistered 2,4-D without considering its endocrine disrupting effects, despite deciding that "[a] repeat 2-generation reproduction study (using the revised EPA protocol) is required to address concerns for endocrine disruption."[17] EPA illogically ignores the existing data on endocrine effects, proceeds with reregistration, and simultaneously determines that there are "concerns for endocrine disruption" that must be addressed. Recent studies, discussed below, establish the dangerous endocrine disrupting effects of 2,4-D and underscore the need for EPA to consider these impacts in its assessment of the health impacts of 2,4-D.

A recent study of several common aquatic herbicides found that 2,4-D has a relatively potent estrogenic effect in fish.[18] Juvenile rainbow trout exposed to 2,4-D for only 7 days had a 93-fold increase in an egg hormone (vitellogenin) that responds to estrogen exposure in fish. The doses used in this study were based upon the recommended application rates for the control of aquatic weeds. These findings indicate an estrogenic effect that is also relevant to mammals, including to humans. However, the immediate relevance of this study is to highlight the ecological risk to fish when 2,4-D is applied to water bodies for controlling weeds. In light of the risk posed by application of 2,4-D to water bodies, EPA should reconsider its decision to allow this use.

Thyroid hormone plays a critical role in the development of the brain. Slight thyroid suppression has been shown to affect neurological development in the fetus adversely, resulting in lasting effects on child learning and behavior.[19] One study showed that thyroid hormone levels are significantly suppressed in ewes dosed with 2,4-D.[20] Similar findings have been reported in rodents, with suppression of thyroid hormone levels, as well as increases in thyroid gland weight and decreases in ovaries and testes weights.[21] 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.

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[17] RED p. 18

[18] Xie L, Thrippleton K, Irwin MA, et al. Evaluation of estrogenic activities of aquatic herbicides and surfactants using an rainbow trout vitellogenin assay. Toxicol Sciences 87(2):391-398, 2005.

[19] 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. Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child. New Eng J Med 1999; 341(8):549-555.

[20] Rawlings NC, Cook SJ, Waldbillig D. 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, 1998.

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

2,4-D causes slight decreases in testosterone release and significant increases in estrogen release from testicular cells.[22] A 2005 study showed that the combination of 2,4-D and testosterone (5-alpha-dihydroxytestosterone) resulted in synergistic increases in the proliferation of prostate cancer cells, including up to a 32-fold increase in activation of these cancer cells.[23] Other studies found that exposure to 2,4-D caused increases in prostate size in laboratory rats.[24]

In rodents, 2,4-D increases levels of the hormones progesterone and prolactin and causes abnormalities in the estrus cycle (similar to the menstrual cycle).[25] Male farm sprayers exposed to 2,4-D had lower sperm counts and more spermatic abnormalities compared to men who were not exposed to it.[26] 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.[27] This increase in birth defects was most pronounced among infants who were conceived in the spring, the time of greatest herbicide use.

Collectively, these studies show that EPA should not allow applications of 2,4-D to waterways where fish may be exposed, since it has been shown both to mimic estrogen in fish at environmentally-relevant doses and to interfere specifically with neurological function in fish. Since hormone systems in fish, rodents, and humans are similar or identical, these studies also indicate a significant potential for 2,4-D to interfere with human hormonal function. Yet EPA failed to take any of this information into consideration when reregistering 2,4-D. Instead, the Agency ignored the existing data and proceeded with reregistration, while also requiring a new study to be done to evaluate "concerns for endocrine disruption."[28]

The FFDCA, as amended by FQPA, required EPA to develop a screening program to determine whether pesticides "may have an effect in humans that is similar to an effect in humans that is similar to an effect produced by a naturally occurring estrogen, or such other endocrine effect...."[29] The Human Health Risk Assessment conducted by EPA states:

> Based on currently available toxicity data, there is evidence of the endocrine-disrupting effects of 2,4-D on mammals. However, no specific measures of such effects have been

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[22] Liu RC, Hahn C, Hurtt ME. The direct effect of hepatic peroxisome proliferators on rat leydig cell function in vitro. Fundamental & Applied Toxicol 30:102-108, 1996.
[23] Kim H-J, Park YI, Dong M-S. Effects of 2,4-D and DCP on the DHT-induced androgenic action in human prostate cancer cells. Toxicological Sciences 88(1):52-59, 2005.
[24] Kim HJ, Kim WD, et al. Mechanism of phenoxy compounds as an endocrine disruptor. J Toxicology Public Health 18:331-339, 2002.
[25] Duffard R, Bortolozzi A, Ferri A, Garcia G, Evangelista de Duffard AM. Developmental neurotoxicity of the herbicide 2,4-dichlorophenoxyacetic acid. Neurotoxicology 16(4):764, 1995.
[26] Lerda D, Rizzi R. Study of reproductive function in persons occupationally exposed to 2,4-D. Mutation Research 262:47-50, 1991.
[27] Garry VF, Schreinemachers D, Harkins ME, et al. Pesticide appliers, biocides, and birth defects in rural Minnesota. Environ Hlth Perspect 104:394-399, 1996.
[28] Reregistration Eligibility Determination for 2,4-D, 18.
[29] 21 U.S.C. § 346a(p)(1).

> attempted. As a result, HED has determined that a repeat 2-generation reproduction study [using the new protocol] is required to address concerns for endocrine disruption [thyroid and immunotoxicity measures].[30]

The RED notes that "[w]hen the appropriate screening and/or testing protocols being considered under the EDSP [Endocrine Disrupting Screening Program] have been developed, 2,4-D may be subject to additional screening and/or testing to better characterize effects related to endocrine disruption."[31] We believe that, to date, EPA has only included the thyroid, androgen and estrogen hormone systems in the program. EPA's congressionally mandated deadline to develop and implement the EDSP passed almost ten years – and to date the Agency has not tested one single chemical.

EPA relies on the hollow excuse that a formal screening program does not yet exist to avoid examining potential endocrine disrupting effects, and as a consequence, it neglects analyzing an entire category of existing scientific studies demonstrating adverse health effects. In fact, the risk assessment omits a group of studies that, taken together, suggest that 2,4-D is an endocrine disrupting chemical, capable of interfering with multiple hormones controlling reproduction and neurodevelopment.

There is precedent for the Agency to consider endocrine disrupting effects in a human health risk assessment in the absence of a final EDSP. For example, in the RED for atrazine, the Agency examined the potential endocrine disrupting effects of atrazine on amphibians, undermining any agency claim that existing studies of the endocrine disrupting effects cannot be considered in its human health risk assessments. Accordingly, given the studies suggesting that 2,4-D has the potential to cause endocrine disrupting effects, EPA should have quantitatively incorporated these studies and these effects in its risk assessment of 2,4-D.

NEURODEVELOPMENTAL TOXIC EFFECTS

2,4-D is neurotoxic, interfering with motor neuron function in studies on mammals and fish. A 2006 study screening a variety of pesticides for neurological toxicity in fish found that sub-lethal exposure to 2,4-D in fish tank water significantly increased brain cell death, disrupted motor neuron growth, and decreased motility.[32] These effects represent specific neurological toxicity and raise additional concerns about the ecological effects of aquatic applications of 2,4-D. In mammals, 2,4-D has been shown to exert related developmental neurotoxicity, including decreased motor activity and Parkinson's like tremors.[33]

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[30] 2,4 D. HED's Revised Human Health Risk Assessment for the Re-Registration Eligibility Decision (RED) Revised to Reflect Public Comments. May 12, 2005, 37.

[31] Reregistration Eligibility Determination for 2,4-D, 81.

[32] Ton C, Lin Y, Willett C. Zebrafish as a model for developmental neurotoxicity testing. Birth Defects Research (Part A) 76:553-567, 2006. http://phylonix.com/BDRA-Ton.pdf

[33] Ton C, Lin Y, Willett C. Zebrafish as a model for developmental neurotoxicity testing. Birth Defects Research (Part A) 76:553-567, 2006. http://phylonix.com/BDRA-Ton.pdf

In young rats, exposure to 2,4-D results in delays in brain development and abnormal behavior patterns, including apathy, decreased social interactions, repetitive movements, tremor, and immobility.[34] 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.[35] This herbicide specifically appears to impair normal deposition of myelin in the developing brain. [36 37 38] 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.

These data provide evidence that postnatal exposures to 2,4-D during the critical period for development of the infant brain raise serious scientific concerns.

MUTAGENIC AND GENOTOXIC EFFECTS

In comments submitted to EPA on the 2,4-D risk assessment, NRDC pointed out that EPA disregarded a number of studies that highlight the mutagenicity and genotoxicity of 2,4-D. EPA responded that it was under no obligation to consider these studies because "positive findings are always confined to samples of 2,4-D formulations and not the pure substance."[39] This response is deficient first because nothing confines EPA only to consider studies that examine the pure substance (that is, the active ingredient). Second, recent studies involving just the active ingredient do indeed confirm the mutagenicity and cytotoxicity findings of the studies ignored by EPA. In light of these points, EPA should not allow the continued use of 2,4-D.

Neither FIFRA nor FFDCA limit EPA's ability to consider formulations in setting tolerances or in making reregistration eligibility determinations. In setting tolerances, FFDCA instructs EPA to "assess the risk of the pesticide chemical residue" based on data about infants' and children's consumption of, special susceptibility to, and the cumulative effects of "such residue."[40] FFDCA defines "pesticide chemical residue" based on the

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[34] Evangelista de Duffard AM, Bortolozzi A, Duffard RO. Altered behavioral responses in 2,4-dichlorophenoxyacetic acid treated and amphetamine challenged rats. Neurotoxicology 16(3): 479-488, 1995.

[35] Bortolozzi A, Evangelista de Duffard AM, Dajas F, Duffard R, Silveira R. Intracerebral administration of 2,4-diclorophenoxyacetic acid induces behavioral and neurochemical alterations in the rat brain. Neurotoxicology 2001 Apr;22(2):221-32

[36] Rosso SB, Garcia GB, Madariaga MJ, Evangelista de Duffard AM, Duffard RO. 2,4-Dichlorophenoxyacetic acid in developing rats alters behaviour, myelination and regions brain gangliosides pattern. Neurotoxicology 2000 Feb-Apr;21(1-2):155-63.

[37] Duffard R, Garcia G, Rosso S, Bortolozzi A, Madariaga M, di Paolo O, Evangelista de Duffard AM. Central nervous system myelin deficit in rats exposed to 2,4-dichlorophenoxyacetic acid throughout lactation. Neurotoxicol Teratol 1996 Nov-Dec;18(6):691-6

[38] Konjuh C, García G, López L, de Duffard AM, Brusco A, Duffard R. Neonatal hypomyelination by the herbicide 2,4-dichlorophenoxyacetic acid. Chemical and ultrastructural studies in rats. Toxicol Sci. 104(2):332-40, 2008.

[39] Response to comments June 7, 2005, p. 10

[40] 21 U.S.C. § 346a(b)(2)(C)(i).

FIFRA definition of pesticide, which is "any substance or *mixture of substances*" intended to prevent, destroy, repel, or mitigate any pest or intended to be a plant regulator, defoliant, or desiccant.[41] Under Phase Five of the reregistration, FIFRA instructs that EPA "shall conduct a thorough examination of all data submitted under this section concerning an active ingredient listed under subsection (c)(2) and of *all other available data* found by the Administrator to be relevant."[42] Furthermore, before a pesticide is reregistered EPA must "obtain any needed *product-specific* data regarding the pesticide...and shall review such data within 90 days after its submission."[43] Neither statute limits EPA to consider data only on the "pure substance" or active ingredient of the pesticide. EPA's exclusion of studies that examine the formulations that are in use is contrary to the stated purpose of both FIFRA and the FFDCA - to protect human health and the environment from exposure to these pesticide formulations that are sold in the U.S. and sprayed on our food and other crops.

Furthermore, there are four new studies that confirm the mutagenicity and cytotoxicity of 2,4-D. Two of these were published since the EPA RED was finalized and two were shortly beforehand but were not cited in the risk assessment. Three of these studies examined just the active ingredient 2,4-D, while the third used a commercial formulation that includes 2,4-D. These results must be considered in determining whether users of these products are being exposed to potential toxicity. In one study of genotoxicity, the researchers discovered that pure 2,4-D did have a mutagenic effect in human white blood cells, but only in the presence of human red blood cells, implying that enzymes in the red cells may be activating the chemical.[44] In one recent study, researchers used both pure 2,4-D and a commercial 2,4-D dimethylamine salt to assess the potential for DNA damage in hamster ovary cells.[45] Both of these agents caused a significant, dose-dependent effect indicating genotoxicity. According to the researchers, this study confirms that pure 2,4-D induces DNA damage in mammalian cells "and should be considered as potentially hazardous to humans."

Apart from these new data, the discussion of the carcinogenicity and mutagenicity of 2,4-D that EPA does provide in the risk assessment is wholly inadequate.[46] Although EPA does acknowledge some positive mutagenicity and cytogenicity studies (e.g. in *Drosophila* larvae, in mammalian cell cytogenic 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 formulations are likely to be

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[41] 21 U.S.C. § 321(q) and 7 U.S.C. § 136(u) (emphasis is added).
[42] 7 U.S.C. § 136a-1(g)(1) (emphasis is added).
[43] 7 U.S.C. § 136a-1(g)(2)(B)(i) (emphasis is added).
[44] Soloneski S, González NV, Reigosa MA, Larramendy ML. Herbicide 2,4-dichlorophenoxyacetic acid (2,4-D)-induced cytogenetic damage in human lymphocytes in vitro in presence of erythrocytes. Cell Biol Int. 31(11):1316-22, 2007.
[45] Gonzalez M, Soloneski S, Reigosa MA, Larramendy ML. Genotoxicity of the herbicide 2,4-dichlorophenoxyacetic and a commercial formulation, 2,4-dichlorophenoxyacetic acid dimethylamine salt. I. Evaluation of DNA damage and cytogenetic endpoints in Chinese Hamster ovary (CHO) cells. Toxicol In Vitro 19(2):289-97, 2005.
[46] 2,4 D. HED's Revised Human Health Risk Assessment for the Re-Registration Eligibility Decision (RED) Revised to Reflect Public Comments. May 12, 2005, 29-31.

cytotoxic and mutagenic. For example, Zeljezic and colleague 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.[47] Metabolic activation significantly increased the frequency of chromatid and chromosome breaks. The same researchers reported significant increases in the number of micronuclei and nuclear buds at this dose level. Another study found a significantly higher rate of sister chromatid exchange (SCE) in chick embryos treated with 2,4-D and its isooctyl ester.[48] Madrigal-Bujaidar and colleagues 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.[49] Other researchers have tested 2,4-D in yeast, in transformed hematopoietic cells, and in mouse bone marrow, and have found both cytotoxic and mutagenic effects, including chromosomal breaks, deletions, and exchanges.[50] Tests in *Drosophila* have also demonstrated genotoxicity to both somatic and germ-line cells.[51]

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 reported that treatment of human erythrocytes in vitro with 2,4-D at 250 and 500 parts per million resulted in decreased levels of reduced glutathione, decreased activity of superoxide dismutase, and increased levels of glutathione peroxidase.[52] 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.[53] The authors of this study concluded that 2,4-D is a cytotoxic agent.

Some researchers have hypothesized that some of the studies showing 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.[54] Yet

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[47] Zeljezic D, Garaj-Vrhovac V. Chromosomal aberrations, micronuclei and nuclear buds induced in human lymphocytes by 2,4-dichlorophenoxyacetic acid pesticide formulation. Toxicology 200:39-47, 2004.
[48] Arias E. Sister chromatid exchange induction by the herbicide 2,4-dichlorophenoxyacetic acid in chick embryos. Ecotoxicol Environ Saf 55(3):338-43, 2003.
[49] Madrigal-Bujaidar E, Hernandez-Ceruelos A, Chamorro G. 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, 2001.
[50] Venkov P, Topashka-Ancheva M, Georgieva M, Alexieva V, Karanov E. Genotoxic effect of substituted phenoxyacetic acids. Arch Toxicol 74:560-6, 2000.
[51] Tripathy NK, Routray PK, Sahu GP, Kumar AA. Genotoxicity of 2,4-dichlorophenoxyacetic acid tested in somatic and germ-line cells of Drosophila. Mutat Res 319(3):237-42, 1993.
[52] Bukowska B. 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, 2003.
[53] Tuschl H, Schwab C. Cytotoxic effects of the herbicide 2,4-dichlorophenoxyacetic acid in HepG2 cells. Food Chem Toxicol 41:385-393, 2003.
[54] Holland NT, Duramad P, Rothman N, Figgs LW, et al. 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, 2002.

several important studies used the pure formulation of 2,4-D. Furthermore, from a practical and legal viewpoint, the critical issue relates to the cumulative risk to the end user, and therefore EPA should take this concern seriously and act to protect users adequately from the toxicity of the end product. Instead, the final risk assessment concludes only that "the possibility of genotoxicity for 2,4-D cannot be ruled out," but fails to actually incorporate this information into the risk assessment.[55]

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 1,700 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 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.[56] 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.[54] 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 non-Hodgkin's lymphoma (NHL) in humans. Unfortunately, EPA failed to mention any of this information in its risk assessment of 2,4-D, thereby failing to assess fully the risk of cancer in humans from this exposure and failing to protect humans from this risk adequately.

EXPOSURES

The FQPA 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."[57] 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).[58] 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 agricultural uses. Furthermore, the

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[55] 2,4 D. HED's Revised Human Health Risk Assessment for the Re-Registration Eligibility Decision (RED) Revised to Reflect Public Comments. May 12, 2005, 30.
[56] Figgs LW, Holland NT, Rothmann N, Zahm SH, et al. Increased lymphocyte replicative index following 2,4-dichlorophenoxyacetic acid herbicide exposure. Cancer Causes Control. 11(4):373-80, 2000.
[57] 21 U.S.C. § 346a(b)(2)(A)(ii)
[58] *Id.*

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 establishing this tolerance for 2,4-D. As outlined extensively in our comments and literature review, there were many deficiencies in the aggregate exposure assessment. Therefore, we incorporate by reference our prior comments and literature reviews on this topic, submitted to EPA on May 7, 2002 and August 20, 2004 respectively. In addition, we provide the following additional, newer information showing that exposure was underestimated with respect to exposure through maternal milk and dermal exposure.

Exposure through maternal milk

There is evidence of exposure to 2,4-D through maternal milk. As explained in our comments, prior to completion of 2,4-D reregistration, research revealed that 2,4-D is excreted in maternal milk, thereby resulting in potentially significant exposures to the nurseling. Researchers detected 2,4-D residues in stomach content, blood, brain and kidney of 4-day-old neonatal rats breast-fed by 2,4-D exposed mothers.[59] When maternal exposures stopped, the chemical continued to be excreted in maternal milk for a week. Despite knowing about this research and this route of exposure, EPA failed to include any lactational exposure in its aggregate risk assessment.

Since the completion of 2,4-D reregistration, additional studies have been published that confirm the lactational exposure and identify adverse effects in the offspring. One 16-day postnatal study found that maternal doses as low as 15 mg/kg/day resulted in significant decreases in body weight gain among rat pups.[60] The 2,4-D accumulated by about 1.6-fold in maternal milk and resulted in alterations in the nutritional content of the milk itself. For example, the fat content of the milk dropped significantly, with a particular reduction in beneficial polyunsaturated fatty acids. Exposure to 2,4-D also caused alterations in the content of some proteins in maternal milk. This study is especially relevant in light of EPA selecting a no observable adverse effect level (NOAEL) of 25 mg/kg/day for short-term (1-30 days) oral exposure. This new study found adverse effects on breastmilk composition and on bodyweight in offspring at doses as low as 15 mg/kg/day. Therefore, EPA should redo the short-term oral risk assessment using 15 mg/kg/day as a lowest observable adverse effect level (LOAEL), rather than 25 mg/kg/day for the NOAEL.

Other recent studies further support the need to consider lactational exposure to 2,4-D in the aggregate risk assessment. Neonatal rats exposed lactationally to 2,4-D in maternal milk showed significant indications of oxidative stress in certain regions of the brain,

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[59] Sturtz N, Evangelista de Duffard AM, Duffard R. Detection of 2,4-dichlorophenoxyacetic acid (2,4-D) residues in neonates breast-fed by 2,4-D exposed dams. Neurotoxicology 2000 Feb-Apr;21(1-2):147-54.
[60] Sturtz N, Bongiovanni B, et al. Detection of 2,4-dichlorophenoxyacetic acid in rat milk of dams exposed during lactation and milk analysis of their major components. Food and Chem Toxicol 44:8-16, 2006.

including the midbrain, striatum, and prefrontal cortex.[61] Lactational exposure to 2,4-D also alters neural development in certain regions of the brain in developing rat pups.[62] This same group of researchers recently published another study showing alterations in brain enzyme levels and neurotransmitters in baby rats exposed to 2,4-D through maternal milk.[63]

Therefore, EPA inappropriately omitted lactational exposure to 2,4-D from the aggregate risk assessment.

Underestimated dermal absorption

In the final risk assessment, EPA used a dermal absorption factor of 10 percent, based on a 1974 study of six adult men. That study showed that 3.4 to 8.2 percent of 2,4-D applied to the skin was absorbed in these six men. Extensive evidence now shows that applying such a low dermal absorption rate is inappropriate, especially considering the synergistic effects of other exposures. Furthermore, several newer studies since the completion of 2,4-D reregistration underscore the fact that EPA's use of dermal absorption factor of 10 percent in the aggregate risk assessment is woefully insufficient.

In 2007, Brand et al studied the interactive effects of alcohol consumption, topical sunscreen application, and exposure to 2,4-D in rats.[64] Alcohol consumption increased skin penetration of 2,4-D by between 1.9 to 2.5 fold; sunscreen further enhanced penetration of 2,4-D by up to an additional 2.9 fold. In fact, a combination of ethanol consumption and sunscreen application acted additively to enhance markedly skin penetration of 2,4-D. This study confirms prior research showing that alcohol consumption and use of commercial sunscreens enhance 2,4-D penetration.[65] [66] [67]

Prior research also demonstrated that the presence of insect repellent containing DEET on the skin significantly enhances absorption of 2,4-D. For example, one study demonstrated 14 percent palmar absorption of 2,4-D after applying DEET to the skin.[68]

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[61] Ferri A, Duffard R, Evangelista de Duffard AM. Selective oxidative stress in brain areas of neonate rats exposed to 2,4-dichlorophenoxyacetic acid through mother's milk. Drug Chem Toxicology 30:17-30, 2007.
[62] Garcia G, Tagliaferro P, et al. Study of tyrosine hydroxylase immunoreactive neurons in neonate rats lactationally exposed to 2,4-dichlorophenoxyacetic acid. NeuroToxicology 25:951-957, 2004.
[63] García GB, Konjuh C, Duffard RO, Evangelista de Duffard AM. Dopamine-beta-hydroxylase immunohistochemical study in the locus coeruleus of neonate rats exposed to 2,4-dichlorophenoxyacetic acid through mother's milk. Drug Chem Toxicol. 29(4):435-42, 2006.
[64] Brand RM, McMahon L, et al. Transdermal absorption of the herbicide 2,4-dichlorophenoxyacetic acid is enhanced by both ethanol consumption and sunscreen application. Food and Chemical Toxicology 45:93-97, 2007.
[65] Pont AR, Charron AR, Brand RM. Active ingredients in sunscreens act as topical penetration enhancers for the herbicide 2,4-dichlorophenoxyacetic acid. Toxicol Appl Pharmacol 195:348-54, 2004.
[66] Brand RM, Charron AR, Dutton L, Gavlik TL, et al. Effects of chronic alcohol consumption on dermal penetration of pesticides in rats. J Toxicol Environ Health A 67(2):153-61, 2004.
[67] Brand RM, Spalding M, Mueller C. Sunscreens can increase dermal penetration of 2,4-dichlorophenoxyacetic acid. J Toxicol Clin Toxicol 40(7):827-32, 2002.
[68] Moody RP, Wester RC, Melendres JL, Maibach HI. 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, 1992.

Because it is highly likely that people will be exposed to combinations of sunscreen, DEET, and 2,4-D, and also possible that homeowners who apply 2,4-D-containing products may do so after consuming alcohol, comments to EPA urged the Agency to consider these effects in the risk assessment. Despite these public comments, EPA provided a cursory and highly deficient response, suggesting that "One means of dealing with this particular issue would be to add a statement to the label of such products [sunscreens, DEET, etc.] informing users of such products that use may enhance the dermal absorption of various substance [sic] encountered in everyday live [sic], including pesticides."[69]

EPA does not have the authority to add label directions to sunscreens or alcohol, but EPA is certainly responsible for protecting the public from excessive and dangerous skin absorption of 2,4-D. To that end, EPA could either require label warnings on products containing 2,4-D (advising applicators to avoid using sunscreen, DEET, or alcohol prior to applying the product) or account for dermal exposure to 2,4-D occurring in combination with those products that enhance absorption. EPA's response to public comment acknowledges the interactive effect on absorption of 2,4-D, yet utterly fails in its responsibility to protect people from this effect.

Another problem with EPA's reliance on the 1974 study of six adults to estimate dermal absorption was the study's failure to use any form of occlusion over the 2,4-D. Therefore, the effect of 2,4-D soaking into clothing or covered by clothing or gloves after skin exposure was not adequately assessed.

Occlusion can significantly enhance skin absorption of dermally-applied materials.[70] A laboratory study focusing on rubber gloves of the types commonly worn by farmers showed that these gloves were highly permeable to 2,4-D when there was simultaneous exposure to DEET and sunlight.[71] Once the 2,4-D penetrates the glove, the chemical would be occluded against the bare skin and absorption would be increased.

These concerns are not merely academic. A Canadian study published in 2005 revealed an association between non-Hodgkin's lymphoma among farmers and a history of simultaneous exposure to phenoxy herbicides, along with use of DEET and rubber gloves.[72] This study suggests that 2,4-D and related chemicals may be penetrating the skin even when gloves are worn; this study also suggests that the skin penetration could be linked to subsequent development of non-Hodgkin's lymphoma in humans.

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[69] EPA. 2,4-D: Response to Public Comments. December 16, 2004, p. 7

[70] 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. J Toxicol Environ Health A. 2003 Jan 24;66(2):133-51.

[71] Moody RP, Nadeau B. Effect of the mosquito repellent DEET and long-wave ultraviolet radiation on permeation of the herbicide 2,4-D and the insecticide DDT in natural rubber gloves. Am Industrial Hygiene Assoc Journal 53:436-441, 1992.

[72] McDuffie HH, Pahwa P, Robson D, Dosman JA, Fincham S, Spinelli JJ, McLaughlin JR. Insect repellents, phenoxyherbicide exposure, and non-Hodgkin's lymphoma. J Occup Environ Med 47(8):806-16, 2005.

CONCLUSION

The body of science surrounding 2,4-D underscores the dangerous nature of the pesticide. There is substantial evidence pointing to its endocrine disrupting effects, mutagenicity, and neurotoxicity. Furthermore, data show that EPA underestimated the aggregate impacts by ignoring lactation exposures and failing to consider combined exposures to 2,4-D with any combination of sunscreen, DEET, sunlight, and gloves.

As a result of EPA's actions, NRDC's members and their children are being exposed to unsafe levels of 2,4-D and will continue to be as long as the 2,4-D registrations and food tolerances challenged in this petition remain in effect. We therefore request that EPA expedite its consideration of this petition in every way possible. If EPA intends to solicit public comment before making a decision on this petition, we request that the Agency do so promptly. EPA's past history of significant delay in responding to pesticide petitions and tolerance objections filed by NRDC constitutes a pattern and practice of unlawful agency inaction that harms NRDC and its members.

Respectfully submitted,

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