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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-Dichlorophenoxyacetic acid Risk Assessment; **Docket ID No. OPP-2004-0167**
Submitted electronically to opp-docket@epa.gov

We submit the following comments on behalf of:

Advocates for Environmental Human Rights;
Agricultural Resources Center;
Alaska Community Action on Toxics;
Alliance for Healthy Homes;
Beyond Pesticides;
Breast Cancer Fund, The;
California Safe Schools;
Californians for Alternatives to Toxics;
Cancer Action New York;
Citizens' Campaign for the Environment;
Citizens' Environmental Coalition;
Clean Water Action;
Coalition for Alternatives to Pesticides, The;
Coalition for Environmentally Safe Communities.
Coalition for Health, Environment and Economic Rights;
Colorado Pesticide Network;
Connecticut Coalition for Environmental Justice;
Defenders of Wildlife;
Ecology Center;
Environmental Research Foundation;
Farmworker Justice Fund, Inc.;
Grassroots Coalition;
Grassroots Environmental Education;
Informed Choices;
Institute for Agriculture and Trade Policy;
Jack B. Richman Environmental Coalition;
Maryland Pesticide Network
Michigan Environmental Council
Minnesota Center for Environmental Advocacy
National Center for Environmental Health Strategies, Inc.;
Natural Resources Defense Council;
New Jersey Environmental Federation;
New York Public Interest Research Group;
No Spray Coalition, Nashville;
Northeast Organic Farming Association of New Jersey
Northwest Coalition for Alternatives to Pesticides;
Pesticide Action Network North America;
Pesticide Free Zone Campaign
Roseland Organic Farms;
Safer Pest Control Project;
Students for Bhopal;
TEDX, Inc. (The Endocrine Disruption Exchange);
Texans for Alternatives to Pesticides;
Toxics Action Center;
Washington Toxics Coalition;
Women's Voices for the Earth;
World Wildlife Fund;
Wyoming Outdoor Council;
(See page 13 to 16 for signatures.)

Our organizations have significant concerns about the EPA human health and ecological risk assessment for 2,4-D. In particular, we request the following of the Agency in these comments:

1. **Toddler Exposure:** EPA is requested to fill the data gap on child exposure, reverse its deviation from typical procedure, provide currently assessed MOE, generate a worst-case risk assessment not using a 7 day average.

2. **Aggregate Exposure:** EPA is requested to complete an assessment for toddlers/children.

3. **The Rat As Predictor of Toxicity to Humans:** EPA is requested to use intermediate approach between the rat and dog and not to choose an exceptionally insensitive species (rat) on which to base the human health risk assessment.

4. **Farmworker and Farmworker Children Assessments:** EPA is requested to either restrict usage to only those crops where hand labor is not employed, or address the problems with the 8-hour work day assumption, and perform additional dermal risk to farmworkers.

5. **Occupational/Residential Dermal Absorption:** EPA is requested to consider range of results of Moody et al 2002 study, and require not recommend additional mitigations.

6. **Cumulative Risk Assessment:** EPA is requested to consider common modes of toxicity of phenoxy herbicides contained in 2,4-D product formulations.

7. **Weed and Feed Products:** EPA is requested to cancel all registered uses of these products.

8. **Ecological Risk Assessment:** EPA is requested to propose risk mitigation measures for identified ecological risks to birds and mammals, particularly for granular products such as the cancellation of all registered uses of weed and feed products.

9. **Dioxins:** EPA is requested to withhold reregistration until all risk of dioxin production and exposure is removed.

10. **Data Gaps:** EPA is requested to withhold reregistration until all data gaps and forthcoming information are received, assessed, and open for public comment.

11. **Carcinogenicity:** EPA is requested to use the weight of evidence and classify 2,4-D as a Class C 'possible human carcinogen.'

Toddler Exposure

In this phase of the risk assessment process, EPA has stated that:

"The 2,4-D risk assessment team decided that the previous approach would greatly overestimate the short term toddler risk because the short term endpoint was based upon maternal effects that would only occur after several days of exposure." (P. 35 OPP-2004-0167-0004.)

EPA should not deviate from its standard practice just to make the numbers work out favorably to the reregistration of 2,4-D. If standard or *typical* practice is to use the short term endpoint

along with the maximum Turf Transferable Residue (TTR) value to assess short term risk for toddlers playing on treated turf, then this should not be changed simply because the MOE is unfavorable to the registrant(s). The very reason for using the maximum TTRs with a short-term endpoint was to make up for a lack of data.

The Agency should call this what it is: a serious data gap on acute and short-term exposure data relevant to children and a deficiency in EPA's data on the short-term health effects relevant to children. It appears the Agency is trying to cobble together a proxy for this data gap by using effects in the adult (maternal) that it claims would not occur with shorter-term exposure. Further, EPA justifies not calculating the acute risks for toddlers using the more protective acute NOEAL of 67mg/kg/day because the maximum TTR rate was used rather than considering how it may decrease through dissipation, which would lead to a lower TTR. We request the Agency provide the calculations that show that the trade-off is justified.

It appears to us that the short-term toddler risk may in fact be underestimated because of the use of an adult maternal endpoint and the averaging of TTRs over 7 days. Using the adult maternal endpoint with maximum TTRs the Agency found a corresponding toddler exposure of MOE 1000. To now say that the adult maternal effects based on several days of exposure is MORE representative of toddler exposure has an inadequate basis. Studies have repeatedly shown that child exposure is among the highest exposure possible, not only in terms of the intake of the chemical through various pathways due to proximity to the ground, breathing zone, hand to mouth behavior, and patterns of play but also in terms of proportional impact of that exposure relative to body weight. Children's developing organ systems are more vulnerable and less able to detoxify toxic chemicals.[1]

On the whole, the Agency's reasoning for adjusting acute and short-term exposure for children from turf treated with 2,4-D is quite confounding. At this stage, the Agency should be discussing mitigation measures to lower children's exposure and risk (MOE 1000 sits too far on the edge of acceptability), rather than changing its practice of using maximum TTRs and "rerunning the numbers," or more accurately, explaining away the previous numbers.

On this we ask the Agency:

1. By how much does the Agency think the approach overestimated risk?
2. What is the current MOE for toddlers? Under Section 4.4.2.3 of the revised HED risk assessment (OPP-2004-0167-0080) the Agency states that toddler exposure is overestimated but does not reconfigure the MOE. How can an accurate assessment be made for aggregate exposure including dietary and water?
3. What is the aggregate exposure for children?
4. What data does the Agency have to assume that granular formulations would result in less or equal exposure, compared to liquid formulations?
5. The rat developmental study showed effects after several days of exposure. Based on that the Agency is averaging a 7-day exposure scenario. How does the Agency come to the conclusion that using an average of 7 days (versus a median, for example) is comparable to several (i.e. at least 3) days exposure for protecting children? By averaging over 7 days, the peak exposure is eliminated.

We also request the Agency:

6. Acknowledge the lack of a relevant short-term study for the infant and issue a data call-in for such a study that could help to shed light on this important exposure scenario.
7. Generate a worst-case risk assessment in which the exposure is not averaged over a week, but is instead compared to the 25 NOAEL.

Aggregate Exposure

Previous comments by Beyond Pesticides, et al. criticized the Agency for neglecting to include in the aggregate risk assessment other important areas of exposure from air drift, migration of contaminated soil, indoor air exposures from residential use, and "take home" exposures from agricultural uses. The Agency's response that, "The resulting maximum doses (assuming a toddler body weight of 15 kg) from these [indoor air from treated turf] exposures are 0.00067 mg/kg/day for floor contact, 0.002 mg/day for table top contact and 0.00045 mg/kg/day for floor dust ingestion. Comparison of these doses with the short-term endpoint of 25 mg/kg/day for decreased bodyweight gain yields MOEs that range from 12,500 to 56,000" is erroneous. The Agency appeared to misunderstand the point. Beyond Pesticides et al's request is that these additional mg/kg/day exposures as well as the other exposures be calculated not on their own in comparison with the short-term endpoint but rather in the aggregate exposure risk assessment. There appears to be no mechanism for degradation when the residues are dry and away from soil microbes in homes where 2,4-D is tracked in.

In Phase 4 of 6 of the risk assessment process HED conceded that it would not do an aggregate exposure on toddlers since exposure to treated lawns found MOE 1000 and therefore any additional dietary and water exposure would clearly be of concern to the Agency. Now that the Agency claims the MOE was overestimated (although it fails to give the new MOE), it does not appear the Agency completed an aggregate exposure that includes dietary and water. We demand the Agency calculate the numbers and not proceed with the reregistration of 2,4-D based on weak verbal assumptions on the risk and exposure.

The Rat As Predictor of Toxicity to Humans

In discussing the issue of assessing human health risk on the basis of rodent studies and not on the toxicity of 2,4-D in dogs, the Agency states:

"While there is definitely substantial evidence that the dog has a lower clearance capacity for organic acids than what is predicted using allometric scaling, although the mechanism to explain the difference is not clear, it is not considered an unique, species-specific, mechanism and therefore, the data on the dog cannot be ruled out. However, the difference in the elimination pattern among dogs and other mammalian species persuaded HIARC that the rat was a better predictor than the dog of the potential toxicity of 2,4-D to man." (OPP-2004-0167-0023.)

In this explanation, the Agency simply defers to HIARC without further explanation and ignores the fact that the rat is NOT an accurate representation of elimination patterns in humans. In previous comments submitted by Beyond Pesticides, et al. we state, "...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." This argument was ignored and sidestepped by the Agency. Humans appear to be more sensitive than rats, albeit less sensitive than dogs. EPA is choosing an exceptionally insensitive species on which to base the assessment.

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 contention that the human resembles the rat. The table below summarizes the plasma half-life of 2,4-D in the rat, the human, and the dog.

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

Further, an important recently published finding of the rat genome showed that rats have detoxification mechanisms that do not exist in people, augmenting the argument that rats are less than ideal animals to model human toxicity.[2] (See Appendix 2.)

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.

Farm worker and Farm worker Children Assessments

We are pleased with the Agency's decision to require all wettable powder formulations be packaged in water-soluble bags. We question why the Agency did not respond to the request that the Agency require all liquid formulations to 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, and request the Agency to please also address this additional concern.

8-hour Farm Worker Work Day Assumption. In pages 3-5 of HED Responses to public comments (OPP-2004-0167-0090), the Agency refers to two recent biomonitoring studies. These studies contain important information but are inadequate to support EPA's conclusions. MOEs calculated from the Farm Family Exposure Study are four-day averages and do NOT represent peak acute exposures. The Agricultural Health Study included analyses of applicators' urine before, one day after and three days after application. The very small number of children (n-9) analyzed, however, provided post application samples two days after application. There was apparently no immediate post-application analysis – again an omission of the peak exposure period.

On page 5, EPA responds to Beyond Pesticides, et al. criticism about the underlying assumption of an 8-hour work day by suggesting that "are primarily made to crops" that do "not involve much hand labor." However, this means that 2,4-D is and can be used on some crops that do employ field workers. Therefore, EPA should either restrict usage to only those crops where hand labor is not employed, or address the problems with the 8-hour work day assumption.

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.[3] This study showed that many agricultural workers work 10 to 12 hour shifts. Interestingly, however, in its recent amendment to the Worker Protection Standard, in response to a grower request, EPA has chosen to allow the use of the glove liners for 10 hours per day, on the grounds that farmworkers work 10 hour shifts and need the glove liners for their entire workday. 69 Fed.Reg. 53,341 (Sept. 1, 2004). Having elected to use a 10-hour workday for farmworkers in one regulatory context, EPA can hardly justify using an 8 hour workday in this area. Indeed, if there is any basis for making such a distinction, EPA has not identified it.

While EPA has divided its analysis of worker exposure into handler and post-application exposures, it has failed to take into account exposures through drift to workers in adjacent fields (which may or may not involve label violations). Thus, in fully evaluating worker exposures, whether the pesticide is used on grain crops or hand labor crops such as sweet corn, EPA must fully evaluate the risks of exposure to workers laboring in nearby fields through drift. We expect to hold the Agency to its promise that, "Any changes to exposure assessment procedures that come out of this review of NRDC's objections [to various tolerance actions] will be considered as EPA completes the 2,4-D RED" (p. 6).

Dermal absorption. In page 7 of HED Responses to public comments (OPP-2004-0167-0090) EPA responds to the criticism of the Agency's use of the dermal absorption rate of 5.8% because it did not take into account the possibility that individuals may also use sunscreens, repellents or occlusion that increase rates of dermal absorption by stating that field workers "most likely would not be using these potential enhancer product." Yet the Agency fails to give any reference to sources from which that assumption is made.

EPA has underestimated the extent to which workers absorb 2,4-D. Notwithstanding EPA's sweeping assertion to the contrary, it is quite likely that *some* of the nation's 2.5 million hired farmworkers do use sunscreen and/or insect repellent while working with or around 2,4-D. Thus these aids to absorption must be considered. Moreover, in the vast majority of instances, workers will be exposed to 2,4-D when they are sweating, due to the physical exertion required to carry out their tasks, and their skin is hot. As such, they are likely to absorb more of the pesticide than the laboratory rats, because absorption is greater when the skin is wet and when blood vessels close to the surface are expanded.

EPA's acknowledgement of concern about dermal exposure is not adequately mitigated by the "recommendation" that workers wash hands frequently and use chemical resistant gloves. If gloves would provide significant protection for pesticide handlers, they must be required because

otherwise they will not be provided. Generally, if gloves are needed because of exposure to post-application workers, restricted entry intervals should be increased because workers harvesting crops on a piece rate cannot do their jobs effectively wearing gloves. In addition, while handwashing facilities are sometimes available, dermal exposure cannot be fully eliminated in the absence of showering and change of clothes facilities. Such facilities should also be required here, in light of the evidence that 2,4-D may pose a cancer risk to workers.

Occupational/Residential Dermal Absorption

Regarding data from the literature on 2,4-D exposure with and without DEET, the Agency discards the findings of the Moody *et al.,* (1992) study (OPP-2004-0167-0090 p. 5) because of large standard deviations. If the only purpose of statistical analysis is to identify and compare population means, then this approach is correct. However, analyses of human exposure should also take into consideration the range of results, which illustrates variability within a population—an important consideration in assuring that protective measures are inclusive of all potentially exposed individuals. Therefore the dismissal of the findings is inappropriate.

In addressing the dermal absorption of infants and children, Beyond Pesticides, et al. did not claim, as EPA suggested, that infant skin is more permeable (although it may be). However, we did point out that other factors may be very important and have not been considered. Such factors include relative ratios of skin area to body mass and the effect of saliva-moistened hands on pesticide absorption.

We agree with EPA that improved labeling of sunscreen, DEET, etc. to inform consumers of such products that their use may enhance the dermal absorption of various substances including pesticides. Unfortunately, EPA does not have the regulatory authority to require such labeling for sunscreens. EPA does, however have the regulatory authority to require label changes on pesticides. Using the same argument, we would then expect to see pesticide labels carry a similar message—that the hazards of dermal exposure to the pesticide may be increased if exposed individuals simultaneously use products containing 2,4-D.

Cumulative Risk Assessment

In pages 13 of HED Responses to public comments (OPP-2004-0167-0090) EPA justifies the dismissal of cumulative risk assessment for various phenoxy herbicides based on the statement that "HED has no data indicating that they have a common mode of toxic action." HED has not demonstrated that there are data that indicate that there is ***no*** common mode of action. Rather, they only cite that they have no such data. Lack of data cannot be used as data showing no common mode of toxic action. In our previous comments we provide evidence that at the very least lend reason to believe these chemicals have a common mode of toxicity and therefore should warrant a review by the Agency.

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

We ask that the Agency revisit the 2002 study (Cavieres, 2002) that found that exposure of pregnant mice to a common combination of phenoxy herbicides including in drinking water resulted in reduced litter sizes.[4] 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). This conclusion is 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 ingredients that likely have a common mechanism of toxicity and that are found in some of the most commonly used lawn care herbicides.

Weed and Feed Products

Weed and feed products that combine fertilizer and 2,4-D are used excessively because consumers do not recognize that these products are pesticides and therefore make applications of herbicide when fertilizer alone would have been sufficient. We request the Agency to discontinue the registration of residential and commercial use of combined fertilizer and pesticide products.

Studies by the U.S. Geological Survey also show 2,4-D to be the herbicide most frequently detected in streams and shallow ground water throughout the country from home and garden use.[5] Registration of weed and feed products undercuts the Agency's promotion of IPM because it requires a broadcast treatment over the entire lawn area, which spreads the herbicide everywhere instead of just where weeds are present. Use of these 2,4-D products also kills non-target plants and countless mammals and birds, as discussed below. Across the country, municipalities will attest that the maximum number of allowed broadcast applications, two times per year, on turf is not followed because people do not recognize the product as an herbicide and therefore are even less likely to read the label (which is a problem anyway). The label for residential use is typically not enforceable. Given that 7 to 9 million pounds of 2,4-D products like weed and feed are used in the home and garden sector per year the exposure to children, especially but not limited to when it is overused, is unacceptable and not sufficiently addressed in the risk assessment.

In the SRRD Response to comments, the Agency responded to SFDE that, "Labeling changes will be considered as part of the reregistration of 2,4-D." For reasons stated above, we believe stronger action is required by the Agency in order to adequately protect human health and the environment from the negative effects of these 2,4-D products.

Ecological Risk Assessment

The EFED 2,4-D risk assessment concludes that "use of 2,4-D on terrestrial sites presents the greatest potential risks to: (1) non-target terrestrial plants (2) mammals, and (3) birds" (p.1). However, the preliminary risk reduction options for 2,4-D do not adequately address the identified risks.

2,4-D has been shown to have negative impacts on a number of animals. In birds, 2,4-D exposure reduced hatching success and caused birth defects.[6] 2,4-dichlorophenol, a breakdown product of 2,4-D, is extremely toxic to earthworms, 15 times more toxic than 2,4-D itself.[7] The herbicide also has negative effects on a range of beneficial insects. It reduces offspring numbers in honeybees, kills predatory beetles and ladybug larvae.[8] This reduction in ladybug numbers caused an increase in aphids, a major "pest", in oat fields.[9] Consumption of plants treated with 2,4-D has also been reported to kill cattle and horses and 2,4-D can also indirectly affect many wild mammal species, including moose, gophers, and voles, by damaging or killing plants they rely on for food.[10]

We are particularly concerned about the ecological risks identified by the agency for lawn uses of granular 2,4-D products. In the ecological risk assessment, an acute risk quotient of 2.5 (exceeding the Agency's level of concern) is calculated for small birds feeding on turf/lawn following a broadcast granular application (p. 487). In addition, acute risk quotients of 2.4 and 1.0 (again exceeding the agency's level of concern) are calculated for small and medium sized mammals feeding on turf/lawn following a granular broadcast application (p. 533).

The Agency's own estimates show that applications by homeowners to lawns in weed and feed products that also contain fertilizer is the second largest use of 2,4-D, accounting for 12 percent of the total use (p. 10). Yet, in the Agency's preliminary risk reduction options there is no mention of how the Agency proposes to reduce the ecological risks from these broadcast granular lawn applications. Neither does the Agency explain how it can effectively reduce the ecological risks to birds and mammals without addressing home lawn 2,4-D fertilizer applications. As proposed, the preliminary risk reduction options are inadequate. Reducing the risks from homeowner granular products is essential.

Dioxins

In the SRRD Response to public comments the Agency states that, "registrants have indicated to the Agency that the manufacturing process for 2,4-D has been refined to minimize the likelihood that dioxin will be formed. This issue will be further addressed in the reregistration eligibility decision (RED) document." At phase 5 of 6, we expect this information to be forthcoming and open to public scrutiny immediately. The epidemiological and experimental literature on dioxin (previously submitted to the Agency) link dioxins from 2,4-D with immune cancers.

This refinement of the manufacturing process is described in a footnote in Canada's Pest Management Regulatory Agency's recent Proposed Acceptability for Continuing Registration (PACR05-01). Evidently 2,4,5-trichlorophenol was no longer incorporated into the herbicide manufacturing (this is a precursor for 2,4,5-T, which was banned decades ago). However, polychlorodibenzodioxins are formed during phenoxy herbicide manufacturing, with higher-

chlorinated congeners and furans being produced at increased temperature.[11] Normally, dioxins with 2 or 3 chlorine atoms will be formed in the manufacture of 2,4-D, but in some jurisdictions only dioxins with four or more chlorine atoms are assessed. We ask that all dioxins reasonably expected to be contaminating 2,4-D products (i.e. with two or more chlorine atomes) be analysed independently. This should be done in off-the-shelf products and in environmental samples where accumulation may have occurred over the years (e.g. on and adjacent to golf courses or areas known for high lawn pesticide use). Additionally, we ask that reregistration be withheld until contamination with recognized persistent, bioaccumulative, toxic, man-made chemicals is thoroughly understood and dealt with.

Data Gaps

EPA has identified several areas where further data and analysis is forthcoming. We expect that the Agency will withhold re-registration until all data gaps are filled, assessed and open for public comment in order that the final reregistration be complete.

Carcinogenicity: EPA Fails To Consider Weight of Evidence

EPA has announced that that it will follow the latest 1999 draft of its Cancer Risk Assessment Guidelines to evaluate the carcinogenic risks of the chemicals regulated. In the Agency's response to Beyond Pesticides et al comments, we see no evidence that a true "weight of the evidence" is being used.

EPA's July 1999 'Draft Guidelines for Carcinogen Risk Assessment', Sec. 2.6.2. explains a Class D classification. "Not classifiable as to human carcinogenicity [Class D]: This descriptor is used when available data are judged inadequate to perform an assessment. This includes a case when there is a lack of pertinent or useful data or when existing evidence is conflicting, e.g. when evidence is suggestive of carcinogenic effects, but other equally pertinent evidence does not confirm a concern."

Objective, peer-reviewed published literature on 2,4-D's carcinogenicity and mutagenicity, which we summarized in previous comments (and augment in Appendix 1 of these comments), show that neither case above applies - there is not a lack of data; and the published data, overall, is far from being "equally" conflicting. Thus, at a minimum, 2,4-D should be classified as a Class C 'possible human carcinogen.'

Financial dependence and resulting subjectivity is the most obvious bias in science. Scientists receiving federal funding are obligated to perform objective science (42 CFR 50.601-7). Objective, or unbiased, science should include measures that ensure the data is independent and free of financial conflict-of-interest. Has the Agency considered the backgrounds of the authors of the studies that have negatively associated 2,4-D with carcinogenicity and then evaluated the data based upon weight of evidence?

In contrast, we argue that the studies the Agency appears to be relying on to keep 2,4-D in the limbo state of a Class D classification are of decrepit quality and far from objective. Many of the authors of the studies with negative findings exhibit at least some financial dependence on the

party with a clear financial interest in their product being found noncarcinogenic. Furthermore, many of the negative studies would fail to pass even the most rudimentary peer-review to get published. The few industry studies that have managed to be published have been included in our assessment of the overall preponderance of the evidence that 2,4-D is mutagenic.

2,4-D has been studied by scientists for 56 years or more and now has one of the largest compiled database of independent literature. Beyond Pesticides, et al. submitted a large data set of published studies including both negative and positive findings in previous comments to the Agency so that it could make an honest evaluation based on weight of evidence of 2,4-D's carcinogenicity.

In reviewing this data the Agency concludes that, "There are numerous epidemiology studies on 2,4-D and related chlorophenoxy herbicides, which provide contradictory findings with respect to an association between 2,4-D and the development of soft-tissue sarcoma and non-Hodgkin's lymphoma. These studies have been examined by various experts and panels of experts who have concluded that some of the studies suggest a possible association between 2,4-D exposure and an increased incidence of these tumors in humans and others do not." Yet, the Agency again fails to weigh the evidence of carcinogenicity.

As we detailed in our earlier comments and augment in these comments, each sub-category of published and mutagenicity and carcinogenicity studies shows a preponderance of evidence that 2,4-D is mutagenic. There is a large, robust and diverse set of published studies showing 2,4-D's carcinogenic activity, more than sufficient to establish that the weight of the evidence plainly indicates that 2,4-D is at least a Class C 'possible human carcinogen.' The Agency's statement, "the possibility of genotoxicity for 2,4-D cannot be ruled out" adds testimony to this fact.

The generally accepted concept of mutagenicity and genotoxicity is explained here by the European Commission's Scientific Committee on Cosmetic Products and Non-Food Products, "Mutagenicity refers to the induction of permanent transmissible changes in the amount or structure of the genetic material of cells or organisms. These changes may involve a single gene or gene segment, a block of genes or whole chromosomes. Effects on whole chromosomes may be structural and/or numerical. Genotoxicity is a broader term and refers to potentially harmful effects on genetic material which are not necessarily associated with mutagenicity. Thus, tests for genotoxicity include tests which provide an indication of induced damage to DNA (but not direct evidence of mutation)."[12] A mutagenic or genotoxic event is accepted as a critical stage in the initiation phase of carcinogenesis.

Mutagenicity. The Agency claims that all the data we previously submitted showing 2,4-D to be mutagenic were are all tests of formulations of 2,4-D and therefore could not be included. We disagree with this claim. Many of the studies previously submitted were on 2,4-D in its pure substance, not in formulation with ingredients. We therefore request the Agency to please rereview our submission, in addition to the five additional mutagenicity studies (four of which support positive findings) submitted with these comments in Appendix 1. These abstracts represent a complete literature search, and reveal that 29 studies have reported that 2,4-D is mutagenic, in contrast to only two studies that failed to find mutagenic effects.

Furthermore, we would like to underscore that the Agency's refusal to consider the results of studies that analyze the effects of formulations of 2,4-D found on the retail market in the U.S. is unwarranted. This data should most definitely be included in the Agency's determination of 2,4-D's classification since the reality of public and environmental exposure to 2,4-D is indeed based on the formulated products and not on exposure to the active ingredient alone. The mandate of the Agency to protect public health and the environment requires this.

Epidemiology. The Cancer Risk Assessment Guidelines require the Agency to weigh the clear conclusion of the epidemiology data. In our assessment, the weight of evidence is overwhelmingly positive as to there being an association of 2,4-D with cancers, particularly of the immune system. The only type of cancer for which the published epidemiology data demonstrates both positive and negative findings is for canine malignant lymphoma. Epidemiology is an imperfect measurement. Yet, that is precisely why the Agency's policy is to use the overall weight of evidence in assessing cancer potential and risk.

Collectively, the published studies that inform the mechanism of 2,4-D's carcinogenicity strongly support 2,4-D's carcinogenicity. In particular, the association of 2,4-D and 'immune cancers' derived from the epidemiology studies is robust and shows ways that 2,4-D alters biochemistry to encourage immune system cancer.

We concur that there exists a certain paucity of published controlled experiments on 2,4-D's ability to initiate or promote cancer in test animals. As experiments are the strongest type of evidence, we find this scientific (i.e. published) data gap a problem. Nevertheless, invoking the requirement to use a 'weight of the evidence' approach in evaluating carcinogenicity we expect the Agency to respond to the objective data we submitted, particularly those that indicate that under experimental conditions 2,4-D promotes already implanted cancers during neonatal exposure (Parfieniuk 1993, Sulik 1996). The timing of the dose may be the critical factor in 2,4-D's carcinogenicity and are likely to explain negative results within the experimental data.

Scientists who review 2,4-D's carcinogenicity on balance favor a causative role. In Ibrahim MA, et al. 1991, the panel reached a weight-of-the-evidence conclusion despite having 2,4-D industry-affiliated scientists on that panel. As in all the other categories of evidence, it is mainly the industry's reviewers who tend to conclude that there is insufficient evidence of 2,4-D's carcinogenicity. (See Appendix 1.)

The Agency responded to our previous comments by stating, " Review of the additional studies cited by BP (and not previously considered) indicate that the studies add very little to our understanding of the cancer epidemiology specifically related to 2,4-D." (OPP-2004-0167-0090 p. 11). Thus, the Agency acknowledges that much of the published literature we submitted was not previously brought to EPA's attention by any party, such as the registrants. That the Agency failed to seek out this information on its own during the re-registration process of 2,4-D is a violation of the public trust, especially given that over 30 such studies on mutagenicity alone have been published - many subsequent to the Agency's reviews in 1994 and 1996.

In sum, we insist the Agency classify 2,4-D based upon the weight of the evidence, which would reclassify 2,4-D, at a minimum, as a Class C 'possible human carcinogen.'

Conclusion

In conclusion, we feel the Agency has failed to address many of the serious concerns about 2,4-D and therefore that its attempt at mitigation is far from complete. In these and prior comments we have identified 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, children and farmworkers, nor used a weight of evidence approach to the carcinogenicity of 2,4-D, we feel it is negligent to 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.

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Sincerely,

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APPENDIX 1

Mutagenicity Studies

In analyzing one of the previously submitted low dose mutagenicity findings (Holland et al. 2002), we concede that we may have made an error in our conversion (0.005 mM = 5picoMoles; rather than 5 nM dose) there were still serious adverse effects found which included low body concentrations well within the limits of exposure for workers and potentially the general public.

A new mutagenicity study (Lueken et al. 2004) presented to the Agency herein gives an important clue as to why some studies may have found no mutagenicity. In this case it finds that 2,4-D is mutagenic, but only under conditions of oxidative stress. Oxidative stress is a ubiquitous environment for cells.

Knapp GW, Setzer RW, Fuscoe JC. 2003. Quantitation of aberrant interlocus t-cell receptor rearrangements in mouse thymocytes and the effect of the herbicide 2,4-dichlorophenoxyacetic acid. Environmental & Molecular Mutagenesis 42:37-43.

Abstract: Small studies in human populations have suggested a correlation between the frequency of errors in antigen receptor gene assembly and lymphoid malignancy risk. In particular, agricultural workers exposed to pesticides have both an increased risk for lymphoma and an increased frequency of errors in antigen receptor gene assembly. In order to further investigate the potential of such errors to serve as a mechanistically based biomarker of lymphoid cancer risk, we have developed a sensitive PCR assay for quantifying errors of V(D)J recombination in the thymocytes of mice. This assay measures interlocus rearrangements between two T-cell receptor loci, V-gamma and J-beta, located on chromosomes 13 and 6, respectively. The baseline frequency in four strains of mice was determined at several ages (2-8 weeks of aged and was found to be stable at similar to 1.5 X 10(-5) per thymocyte. Strain AKR, which has a high susceptibility to T-cell lymphomas, did not show an elevated frequency of aberrant V(D)J events. We used this assay to examine the effects of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) on the frequency of these events. Female B63F1 mice, 27 days of age, were exposed to 2,4-D by gavage at doses of 0, 3, 10, 30, and 100 mg/ kg/day for 4 successive days and sacrificed on day 5. Thymus DNA was isolated and examined for illegitimate V(D)J recombination-mediated gene rearrangements. In addition, pregnant mice were exposed to 2,4-D and thymocytes from the offspring examined at 2 weeks of age. No significant increase in aberrant V(D)J rearrangements was found, indicating that under these conditions 2,4-D does not appear to effect this important mechanism of carcinogenesis. [References: 40] Number of References 40 Keywords:

Pavlica M, Papes D, Nagy B. 1991 Jun. 2,4-Dichlorophenoxyacetic acid causes chromatin and chromosome abnormalities in plant cells and mutation in cultured mammalian cells. Mutat Res 263:77-81.

Abstract: The cytotoxic and mutagenic effects of the synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D) on shallot root tip cells and on V79 Chinese hamster fibroblast cells were examined and compared. In shallot root tips 2,4-D caused changes in mitotic activity, as well as changes in chromosome and chromatin structure, and also changes during the cell cycle. 2,4-D also showed mutagenic and cytotoxic effects on V79 cells in culture in concentrations higher than 10 micrograms/ml. The results in both systems (plant and mammalian cells) were in agreement showing mutagenic activity of 2,4-D in the concentration range higher than usually used in establishing plant tissue culture (greater than 5 micrograms/ml).

Gonzalez M, Soloneski S, Reigosa MA, Larramendy ML. 2005 Mar. Genotoxicity of the herbicide 2,4-dichlorophenoxyacetic & a commercial formulation, 2,4-dichlorophenoxyacetic acid dimethylamine salt. I. Evaluation of DNA damage & cytogenetic endpoints in Chinese Hamster ovary (CHO) cells. Toxicol In Vitro 19:289-97.

Abstract: Genotoxicity of the 2,4-dichlorophenoxyacetic acid (2,4-D) and a commercially-used derivative, 2,4-D dimethylamine salt (2,4-D DMA), was evaluated in CHO cells using SCE and single cell gel electrophoresis (SCGE) assays. Log-phase cells were treated with 2.0-10.0mug/ml of herbicides and harvested 24 and 36h later for SCE analysis. Both agents induced significant dose-dependent increases in SCE, regardless of the harvesting time (2,4-D: $r=0.98$ and $r=0.88$, $P<0.01$, for 24 and 36h harvesting times; 2,4-D DMA: $r=0.97$ and $r=0.88$, $P<0.01$, for 24 and 36h harvesting times). Neither test compound altered cell-cycle progression or proliferative replication index ($P>0.05$), but the higher doses of both compounds reduced the mitotic index of cultures harvested at 24 and 36h ($P<0.05$). A 90-min treatment with 2.0-10.0mug/ml 2,4-D and 2,4-D DMA produced dose-dependent increases in the frequency of DNA-strand breaks detected in the SCGE assay, both in cultures harvested immediately after treatment and in cultures harvested 36h later. The doses of 2,4-D and 2,4-D DMA were equally genotoxic in all of the assays. The results indicate that 2,4-D induces SCE and DNA damage in mammalian cells, and should be considered as potentially hazardous to humans.

Lueken A, Juhl-Strauss U, Krieger G, Witte I . 2004 Feb 28. Synergistic DNA damage by oxidative stress (induced by H2O2) and nongenotoxic environmental chemicals in human fibroblasts. Toxicol Lett 147:35-43.

Abstract: Genotoxic combination effects of oxidative stress (induced by H2O2) and eight nongenotoxic environmental chemicals (4-chloroaniline, 2,3,4,6-tetrachlorophenol, lindane, 2,4-dichloroacetic acid (2,4-D), m-xylene, glyphosate,

nitrilotriacetic acid and n-hexanol) were determined in human fibroblasts. Genotoxicity was measured quantitatively by the single cell gel electrophoresis assay. The nongenotoxic chemicals were used in non cytotoxic concentrations. H2O2 was used in concentrations producing low (50 microM) and no cytotoxicity (40 microM). All environmental chemicals acted in a synergistic way with H2O2 except DMSO which effectively inhibited H2O(2)-induced DNA damage. The most effective enhancers were 4-chloroaniline, 2,3,4,6-tetrachlorophenol, m-xylene, and n-hexanol. Synergistic effects of hexanol/H2O2 were still evident at a concentration of 0.09 noec (no observed effect concentration). In contrast to synergistic DNA damage in the cell antagonism was found measuring DNA breakage in isolated PM2 DNA. From the results we concluded that synergisms between H2O2 and nongenotoxic chemicals may be a general phenomenon which is not observed on the level of isolated DNA. [So even allegedly non-genotoxic chemicals are genotoxic, under oxidative damage stress--a ubiquitous condition. That would show EPA and the industry's claim of 2,4-D's non-mutagenicity to be false ...if it weren't already overwhelmingly (~25 to 2) shown to be false!]

Micic M, Bihari N, Mlinaric-Rascan I. 2004. Influence of herbicide, 2,4-dichlorophenoxy acetic acid, on haemocyte DNA of in vivo treated mussel. Journal of Experimental Marine Biology and Ecology 311:157-169.

Abstract: The influence of the herbicide 2,4-dichlorophenoxy acetic acid (2,4-D) on haemocyte DNA of in vivo treated mussels Mytilus galloprovincialis has been investigated by flow cytometry and epifluorescence microscopy. Haemocyte proliferation and atypical flow cytometric DNA histograms were observed in mussels treated with 20 and 100 mug/g of 2,4-D. The stimulation of proliferation by 2,4-D was also obvious by DNA labeling with BrdU followed by FITC conjugated anti-BrdU MoAb visualized by epifluorescence microscopy. An apoptotic sub-G, peak resulted in mussels that were exposed to higher doses of herbicide at 100 and 500 mug/g as well as subpopulation could be detected by flow cytometric analysis. In these experiments morphological changes characteristic for apoptotic cells were looked for by fluorescence microscopy. A low percentage of cells in S as well as in G(2)M phase indicating G1 arrest were detected in haemocytes from these mussels that had survived 4 days of 20 mug/g 2,4-D exposure. In addition, sister-chromatid exchanges (SCE) could be seen with the immumolabelling BrdU method. Thus, in vivo treatment and the subsequent uptake of 2,4-D causes serious genetic consequences and raises concerns regarding the potential overall fitness and health effects in mussel populations.

Nedopytanska N, Kornuta N, Bagliy E. 2004. Promoting and genotoxic effect of 2,4-D in rats. Toxicol Appl Pharmacol 197:202. [Even though PubMed indexes this journal, it does not contain this study. Judging by the title, we assume it has positive findings of mutagenicity. We request the Agency obtain a copy of the study for review.]

Ibrahim MA, Bond GG, Burke TA, Cole P, Dost FN, Enterline PE, Gough M, Greenberg RS, Halperin WE, McConnell E, et al. Center for Risk Analysis, Harvard School of Public Health, Boston, MA. Weight of the evidence on the human carcinogenicity of 2,4-D. Enviro Health Perspect. 1991 Dec; 96:213-22.

Abstract: The phenoxy herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) is widely used to control the growth of weeds and broadleaf plants. We convened a panel of 13 scientists to weigh the evidence on the human carcinogenicity of 2,4-D. The panel based its findings on a review of the toxicological and epidemiological literature on 2,4-D and related phenoxy herbicides. The toxicological data do not provide a strong basis for predicting that 2,4-D is a human carcinogen. Although a cause-effect relationship is far from being established, the epidemiological evidence for an association between exposure to 2,4-D and non-Hodgkin's lymphoma is suggestive and requires further investigation. There is little evidence of an association between use of 2,4-D and soft-tissue sarcoma or Hodgkin's disease, and no evidence of an association between 2,4-D use and any other form of cancer. Scientists on the panel were asked to categorize 2,4-D as a "known," "probable," "possible," or "unlikely" carcinogen or as a noncarcinogen in humans. The predominant opinion among the panel members was that the weight of the evidence indicates that it is possible that exposure to 2,4-D can cause cancer in humans, although not all of the panelists believed the possibility was equally likely: one thought the possibility was strong, leaning toward probable, and five thought the possibility was remote, leaning toward unlikely. Two panelists believed it unlikely that 2,4-D can cause cancer in humans. Publication Types: * Review* Review, Tutorial PMID: 1820267 [PubMed - indexed for MEDLINE]

APPENDIX 2

Rat genome unveiled

Deciphered DNA will boost medical research.
1 April 2004

HELEN R. PILCHER

The humble lab rat is the latest to spill its genetic secrets. The Brown Norway rat (*Rattus norvegicus*) is the third mammal to have its genome sequenced, joining mouse and man. The information should help medical research and further our understanding of evolution.

The sequence will further raise the rat's high-profile in medical research. Over the last century, the rat's image has transformed from plague carrier to indispensable tool in experimental medicine and drug development (see Rat Roll of honour). It is the animal of choice for studying physiology and pharmacology, and is used in fields ranging from cardiovascular disease to space motion sickness.

"The rat is used to mimic just about every aspect of every disease known to man," says pharmacologist John Fozard from Novartis in Basel, Switzerland. Knowing its genetic make-up will help researchers find disease-related genes, and further tease apart how genes and the environment affect health.

The sequence, revealed in *Nature*[1], has about 25,000 genes. Around 90% of these have matches in the mouse and man. This means that almost all of the known disease-related human genes have counterparts in the rat, says Richard Gibbs from Baylor College of Medicine, Houston, Texas, who led the collaborative Rat Genome Sequencing Consortium. By tweaking these, researchers should be able to make better rat models of disease.

It will also aid drug discovery, says Gibbs. Rats are widely used to test drug efficacy and safety. Knowledge of their genome should throw up new targets for drug intervention. "You cannot over emphasize the importance of having a complete database like this," he adds.

Of rats and men

The new information also enables three-way comparisons to be made between the genomes of man, mouse and rat.

Around 10% of the rat's genes are both shared with the mouse and absent in humans, including some that code for smell-related proteins. This may explain rodents' exceptional sense of smell, says geneticist Kerstin Lindblad-Toh from the Broad Institute, Cambridge, Massachusetts.

Rats also have more genes for breaking down toxins than man. This means that rats may be better at removing toxins from their bodies than humans. "It may be more difficult than we'd

thought to use the toxicity of drugs in rats as a guide to their toxicity in humans," says Lindblad-Toh. Rats are still commonly used in such toxicity tests, though researchers are increasingly using tissue cultures instead of animals.

Comparisons also suggest that rats evolved three times faster than humans, since the rat genome is much more diverse than our own.

The mutations that create this diversity probably occurred at random, then stuck around because they gave the rat some evolutionary advantage. "These are examples of where the rat has taken advantage of an evolutionary niche and specialized," says Lindblad-Toh. The rat's genetic diversity may have enabled it to colonize a wide range of habitats all over the world, she adds.

The rat genome may be more diverse than the human one simply because their genes mutate more quickly than our own, says Lindblad-Toh. Or, which is more likely, their shorter life span means they have had a greater number of generations in which to select genetic changes, she says.

The new genome was compiled by combining methods from the human and mouse projects. This produced a high-quality sequence that covers 90% of the rat genome. "This makes it more efficient and thorough than previous genome sequences," says Gibbs.

Gibbs' team is also working to produce the cow, macaque and sea urchin sequences. "This should help us to refine our evolutionary comparisons," he says.

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