Comments on
The Natural Resources Defense Council's
Petition to Revoke All Tolerances and Cancel All Registrations
for the Pesticide 2,4-D

(Docket ID EPA-HQ-OPP-2008-0877)

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Executive Summary

The Industry Task Force II on 2,4-D Research Data ("2,4-D Task Force" or "Task Force") submits these updated comments in response to the November 6, 2008 petition of the Natural Resources Defense Council ("NRDC") requesting EPA to revoke all tolerances and cancel all registrations for the pesticide 2,4-dichlorophenoxyacetic acid ("2,4-D") (NRDC, 2008).

The 2,4-D Task Force comments have been updated to integrate results from the EPA required studies, March 2007 EPA Data Call-in. New information from the F1- extended one generation reproduction study incorporates results for reproductive toxicity, developmental neurotoxicity, developmental immunotoxicity, and endocrine end points including thyroid (Marty *et al*., 2010, MRID 47972101). Additionally, results are included from assays relevant to assessment of 2,4-D's potential endocrine modulating activity conducted under the auspices of the US EPA as part of the ToxCast™ program (Judson *et al*., 2009).

The Task Force respectfully submits that 2,4-D meets the requirements for its continued registration under FIFRA and the continuance of its residue tolerances under the FFDCA and, accordingly, requests EPA to deny NRDC's petition. From 1988 through June, 2005 when EPA issued the 2,4-D Reregistration Eligibility Decision ("RED"), the Task Force and its members submitted to EPA over 500 studies on 2,4-D, virtually all of which met the Agency's FIFRA Good Laboratory Practice ("GLP") standards. The Task Force sponsored and submitted these studies to satisfy FIFRA's reregistration requirements and the requirements of the Food Quality Protection Act of 1996 that the Agency reassess all tolerances under the Act's standard of "reasonable certainty of no harm."

EPA's reregistration team, consisting of 20 Agency scientists and regulatory personnel, over the course of several years carefully and thoughtfully reviewed the Task Force and other 2,4-D studies to prepare, receive public comment on and revise numerous human

and environmental risk assessments on 2,4-D, the end product of which was the Agency's 320 page 2,4-D RED that determined that 2,4-D met FIFRA's and FFDCA standards for registration and which served as the basis for new 2,4-D tolerances that the Agency proposed in draft form on June 6, 2007 and issued in final form on September 12, 2008. In certain cases, EPA's risk assessments identified some uses of 2,4-D where the margin of exposure was inadequate and the Agency required risk mitigation measures, such as protective clothing, as a condition to continued registration. In addition, even though the Task Force had submitted over 500 studies, the Agency required the Task Force to conduct additional studies, such as a new reproduction study and a new developmental neurotoxicity study. These studies are now complete and in review at EPA.

Against the above history, the NRDC filed a petition in November 2008 that summarily claims that the Agency must now cancel 2,4-D registrations and revoke all 2,4-D tolerances, notwithstanding the past efforts of the Task Force and EPA over 17 years to develop and review numerous GLP studies (many of which the Task Force published in peer reviewed scientific journals), and after several years and repeated opportunities for public comment on the Agency's 2,4-D human and environmental risk assessments.

The Task Force requests EPA to deny NRDC's Petition on several grounds, which are set forth in detail below. First, to the extent that NRDC's Petition requests EPA to revoke 2,4-D tolerances, its Petition is too late. NRDC did not comment when EPA in June, 2007 proposed new 2,4-D tolerances to implement the requirements of the June, 2005 2,4-D RED. EPA issued final 2,4-D tolerances in the form of new regulations on September 12, 2007. NRDC did not file objections to the final 2,4-D tolerances within 60 days of September 12, 2007 as provided for under FFDCA § 408(g)(2). Second, the Task Force requests EPA to deny NRDC's petition because it fails to state with any specificity or any particularity how or why EPA's quantitative risk analysis for 2,4-D for human or environmental risks, as set forth in EPA's June, 2005 RED and its supporting documents, is wrong. Thus, a fundamental flaw of NRDC's Petition is that it fails to recognize that FIFRA and FFDCA are risk-based laws and the end-product of EPA's weight of the evidence human and environmental risk assessment paradigm is a number,

a risk quotient, that tells EPA that a margin of exposure or level of concern is adequately protective of man and the environment or that it is not. The NRDC Petition fails to explain how or why EPA's risk quotients for 2,4-D are wrong and for this reason alone EPA should deny the petition.

Even if NRDC's Petition were timely and even if it were to adequately explain how its concerns relate to EPA's quantitative risk assessments for 2,4-D, its claims are wrong that 2,4-D causes estrogenic, neurodevelopmental and mutagenic effects and that the Agency failed to conduct an adequate aggregated exposure assessment. The Task Force's comments review and carefully respond to each claim. The Task Force respectfully submits that the weight of the evidence demonstrates that 2,4-D when used according to its label does not cause estrogenic, neurodevelopmental or mutagenic effects. Additionally, EPA has already considered and addressed NRDC's exposure concerns, as discussed below.

The fundamental weakness of NRDC's petition is that it does not support its claims with a "weight of the evidence" analysis of all studies that have measured a given endpoint, GLP and non-GLP, but instead selectively cites studies that it thinks supports its view. Many of the studies that it cites were run at excessively high doses that bear no relationship to possible levels of human or environmental exposure. In others, the route of administration (*e.g.*, direct injection of 2,4-D into a rat's brain) is not relevant to human or environmental routes of exposure. In other cited studies, it is impossible to tell what test substance is responsible for an effect (*e.g.*, injecting 2,4-D in a rat after injecting it with Phenobarbital).

The Task Force recognizes and appreciates that even with the benefit of over 500 GLP studies, legitimate regulatory questions may exist and that is the reason Congress included FIFRA § 3(c)(2)(B) in the law that authorizes the Agency to require registrants to submit additional studies. EPA's 2,4-D RED and its Data Call-In Notice required 2,4-D registrants to conduct and submit new reproduction and developmental neurotoxicity studies. The Task Force, in close cooperation and consultation with EPA,

addressed both requirements with a state-of-the-art F1-extended one generation reproduction study with 2,4-D. This study is complete (Marty *et al*., 2010) and shows a lack of adverse reproductive findings, endocrine effects, developmental neurotoxicity or developmental immunotoxicity at doses below a toxicokinetically-derived maximum tolerated dose (KMD) considered relevant for risk assessment. The overall NOAEL from this study provides an ample margin of safety, and the extensive parameters evaluated across life stages provide confidence that no data base uncertainty factor is necessary for 2,4-D. Further details regarding the results of the F1-extended one generation reproductive toxicity study will be provided in these comments in response to specific issues in the NRDC claims, *e.g*., results on endocrine testing will be discussed in the context of NRDC's claim that 2,4-D has estrogenic activity.

2,4-D DOES NOT CAUSE ENDOCRINE DISRUPTION:

The weight of the evidence does not support NRDC's claim that 2,4-D is an endocrine disruptor at doses relevant to human risk assessment. The Task Force's recently completed F1-extended one generation reproduction study (Marty *et al.*, 2010) conducted to satisfy EPA's March, 2007 DCI, comprehensively assessed endocrine-related endpoints, and supplemented existing in vivo and in vitro studies that demonstrate that 2,4-D does not affect the endocrine system. The study design and elements included for characterization of potential endocrine-modulating effects were approved by the US EPA for that purpose. Evaluations across life stages included numerous endpoints sensitive to estrogenic, androgenic, anti-estrogenic and anti-thyroid modes of action (MOA) (detailed later in these comments) and these showed no evidence of exposure-related adverse endocrine effects at dose levels below the KMD (Marty *et al*., 2010).

2,4-D DOES NOT CAUSE NEURODEVELOPMENTAL EFFECTS:

The studies cited by NRDC do not provide credible or substantive evidence that 2,4-D causes neurodevelopmental toxicity at exposure levels or routes of administration relevant to humans. A comprehensive recent F1-extended one generation study found no adverse effects on developmental neurotoxicity endpoints (Marty *et al*., 2010), detailed later in these comments.

2,4-D IS NOT MUTAGENIC:

2,4-D acid plus eight different 2,4-D derivatives have been tested in a battery of mutagenicity tests leading to a total of 28 studies submitted in support of reregistration, all of which were negative (non-mutagenic). While EPA acknowledged that some positive mutagenicity studies occur, the weight of the evidence overwhelmingly supports a conclusion of minimal or no concern for mammalian mutagenicity for 2,4-D.

EPA HAS CONSIDERED EXPOSURE THROUGH MATERNAL MILK

EPA has previously considered and rejected NRDC's concern. The Agency has stated that it is aware, as a result of animal feeding studies, using exaggerated doses that 2,4-D may be present in milk (US Federal Register 2005 at 46706, 46735). EPA has previously informed NRDC that its manner of doing risk assessment for infants is protective of any pesticide exposures to infants from human breast milk.

EPA DID NOT UNDERESTIMATE HUMAN DERMAL ABSORPTION

Five published studies using human subjects demonstrate that EPA's dermal absorption factor of 10% was amply protective and higher than the mean absorption rate from the five human studies of 5.7%.

EPA has considered NRDC's exposure concerns and NRDC fails to explain how its concerns would have changed EPA's 2,4-D risk assessment

EPA has previously considered and rejected NRDC's claim that dermal absorption may be enhanced through other factors such as sunscreen, alcohol consumption and insect repellent. Moreover, EPA's 2,4-D RED required personal protective equipment that mitigate these factors. Several well-conducted studies demonstrate that rubber gloves reduce 2,4-D exposure. NRDC's claim that 2,4-D tracked into homes and persists in carpets for up to one year at concentrations of 0.5 μg/gm is at odds with studies reporting average levels of 0.156 and 0.0475 μg/gm in carpet collected in homes of 2,4-D applicators. Moreover, as noted above, NRDC's Petition fails to explain how any of its exposure concerns would have altered or changed EPA's risk assessments.

The Task Force appreciates the opportunity to submit these updated comments.

INTRODUCTION

The Industry Task Force II on 2,4-D Research Data ("2,4-D Task Force" or "Task Force") has incorporated updated comments in response to the November 6, 2008 petition of the Natural Resources Defense Council ("NRDC") requesting EPA to revoke all tolerances and cancel all registrations for the pesticide 2,4-dichlorophenoxyacetic acid ("2,4-D") (NRDC 2008).

The 2,4-D Task Force comments have been updated to integrate results from the EPA required studies, March 2007 EPA Data Call-in. Updated information from the F1 extended one-generation reproduction study incorporates results for reproductive toxicity, developmental neurotoxicity, developmental immunotoxicity, and endocrine disruption end points including thyroid (Marty *et al*., 2010, MRID 47972101). Additionally, results are included from assays relevant to assessment of 2,4-D's potential estrogenic activity conducted under the auspices of the US EPA as part of the ToxCast™ program. (Judson, *et al*.,2009).

The Task Force respectfully submits that 2,4-D meets the requirements for its continued registration under FIFRA and the continuance of its residue tolerances under the FFDCA and, accordingly, requests EPA to deny NRDC's petition.

EPA Should Deny NRDC's Petition To Revoke 2,4-D Tolerances Because It Is Untimely

NRDC's Petition to revoke 2,4-D tolerances comes too late. NRDC has had ample opportunity to comment on and object to the 2,4-D tolerances EPA established in accordance with the June 2005 Reregistration Eligibility Decision ("2,4-D RED" or "RED") (USEPA 2005). FFDCA § 408(g) requires a person to file objections to a regulation establishing a tolerance for a pesticide chemical residue within 60 days after the regulation establishing the tolerance. EPA established the current 2,4-D tolerances by rule under FFDCA § 408(e) on September 12, 2007 and the period for filing objections expired on November 12, 2007 (US Federal Register 2007b).

Following EPA's June, 2005 publication of the 2,4-D RED, on June 6, 2007, EPA published in the Federal Register a proposed rule to revoke old (pre-RED) tolerances for certain pesticides, including 2,4-D, in order to establish new tolerances set by EPA in the pesticides' REDs (US Federal Register 2007a). EPA opened an electronic docket (OPP-2007-0097) and invited public comment by August 6, 2007 on the proposed 2,4-D tolerances. NRDC did not submit comments and on September 12, 2007, EPA published the 2,4-D tolerances in their final form as regulations at 40 C.F.R. § 180.142. (US Federal Register 2007b at 52013). The Agency's final ruled advised that the 2,4-D tolerances were adopted in accordance with the Agency's authority under FFDCA 408(e). (Id. at 52015).

FFDCA § 408(g)(2)(A) provides that "within 60 days after a regulation or order is issued under [408(e)] any person may file objections thereto with the Administrator, specifying with particularity the provisions of the regulation or order deemed objectionable and stating reasonable grounds . . . ." NRDC failed to file comments on EPA's 2,4-D tolerances and failed within 60 days of September 12, 2007 to file objections to the 2,4-D tolerances. It can not now belatedly petition EPA to revoke these tolerances when it failed to either comment on the tolerances when they were proposed or when it failed to timely object to the tolerances in accordance with the § 408(g)(2)(A). With the exception of one 2008 study, NRDC's petition relies on studies that would have been available to it in 2007. Thus, NRDC's Petition does not now raise any issues or data that it could not have raised had it filed a timely petition in 2007.

EPA Should Deny NRDC's Petition Because It Does Not Challenge With Particularity or Specificity EPA's Quantitative Human or Environmental Risk Assessments

NRDC's petition (at page 3) recites the basic requirements of FIFRA and FFDCA: 1) EPA may not register or reregister a pesticide if it causes "unreasonable adverse effects on the environment;"

2) EPA may establish a pesticide tolerance for pesticide chemical residue only if "there is a reasonable certainty that no harm will result from aggregate exposure" to the pesticide chemical residue, and

3) the Agency must explicitly assess the risks of any pesticide residue to infants and children. (NRDC Petition at 3). The Task Force does not dispute NRDC's statement of law. EPA, however, should dismiss NRDC's petition because it fails to challenge with any particularity or specificity a single EPA quantitative risk assessment for a human or environmental population exposed to 2,4-D.

FIFRA and FFDCA are risk-based statutes under which EPA assesses the risk a pesticide or a pesticide tolerance poses to humans and the environment in order to determine if the pesticide or its residue meets the standards of FIFRA and FFDCA. The Agency assesses a pesticide's risks through a well defined and well accepted four step paradigm consisting of the following steps: hazard identification, dose response assessment, exposure assessment and risk characterization. To complete the first two steps, EPA reviews principally mammalian and ecotoxicity studies prescribed by the Agency in 40 CFR Part 158. These studies are conducted by independent laboratories under contract to the pesticide registrant(s), and are conducted under GLP requirements to assure that protocol requirements are complied with, any deviations are identified and their potential impact on study interpretation addressed, and that the reported results are consistent with the raw data. These studies are conducted in order to identify a pesticide's acute, sub-chronic and chronic effects and to determine the dose(s) at which any effects occur and the dose at which the effect does not occur, which is generally termed the NOAEL or no adverse effect level. Derivation of the NOAEL recognizes Paracelsus' 15th century principle that "the dose makes the poison." While the Agency relies principally on the EPA-prescribed studies submitted by the registrant to complete the first two steps, the Agency can and does rely on qualified studies from the open literature. To meet the requirements of the 2,4-D Registration Standard issued by EPA in 1988, the Task Force and its members submitted to EPA over 500 GLP studies on 2,4-D and its derivative salt and ester forms.

When assessing a pesticide's hazard and the dose at which the hazard occurs EPA utilizes an approach it refers to as "weight of the evidence." "The approach requires a critical analysis of the entire body of available data for consistency and biological plausibility." Federal Register Oct. 26, 2007 at 60933, 60937 (US Federal Register 2007c). In making a weight of the evidence assessment, the Agency looks to sufficiency of data, quality of data, evidence of causality, and whether the study corroborates its findings. (Id.) "Weight-of-evidence is not to be interpreted as simply tallying the number of positive or negative studies." (Id.) A fundamental weakness of the NRDC petition is that it selectively cites only the studies that it believes supports its position, regardless of the study's quality or its relevance. The NRDC petition does not undertake to look critically and objectively at all the studies on an endpoint and assess the weight of the evidence.

In determining the dose at which a pesticide may cause a short, intermediate or long term effect, the Agency applies a 10x interspecies extrapolation factor and another 10x factor for intraspecies variation. The FQPA requires the Agency to apply another 10x factor to protect children and infants. EPA reduced the 10x FQPA safety factor for 2,4-D to 1x because "the Agency has no residual concerns for the effects seen in the developmental toxicity studies." (USEPA 2005 at 19). EPA, however, did apply an additional 10x database uncertainty factor to account for the lack of a developmental neurotoxicity study, a relatively new requirement. (Id.) As well, EPA may adjust for factors such as the amount of the pesticide that will be absorbed through the skin.

EPA assesses human exposure to pesticides through inhalation, skin and oral routes. In assessing exposure, the Agency may use data collected from actual exposure studies, or it may calculate exposure based on computer models that conservatively estimate exposure to a pesticide through, for example, the diet or drinking water.

In the fourth and final step EPA divides estimated exposure levels for occupational and residential populations by the dose level which produces no observed adverse effect in the animal studies, as adjusted by the safety factors discussed above. This calculation results in a margin of exposure ("MOE"). EPA typically considers a margin of exposure

above 1,000 to be adequately protective of humans. If an MOE falls below 1,000, EPA will impose a risk mitigation measure and if a risk mitigation measure is not adequately protective then the Agency may require use rate reductions and/or cancel one or more of the pesticide's uses. The counterpart for an environmental risk to terrestrial and aquatic animals and plants is a quotient expressed as Level of Concern ("LOC"). Levels of regulatory concern for environmental effects range from 0.005 to 1. Tables in the 2,4-D RED report the human and environmental risk quotients for the herbicide.

EPA should dismiss NRDC's petition because it fails to challenge a single particular EPA human health or environmental risk quotient in the Agency's 2,4-D RED: that is, it does not challenge a single MOE for an exposed human population or a single LOC for an exposed environmental population. Thus, NRDC must do more than selectively cite studies to claim that 2,4-D presents an endocrine, neurodevelopmental or mutagenic hazard or that EPA underestimated the contribution of a route of exposure. NRDC must explain with specificity and particularity how an alleged effect or an alleged exposure impacts EPA's four-step risk assessment process and how the effect or exposure would change a particular MOE or LOC in EPA's RED. At the end of the day, NRDC's Petition falls substantially short of this mark and for this reason alone, EPA should deny the Petition.

2,4-D Is Not Present in Fifty Percent of Water Samples

The NRDC petition starts off on shaky scientific grounds. The Petition's Introduction (second paragraphs) claims that "*2,4-D is found as a contaminant in about half of all surface water samples across the U.S. … .*" NRDC cites as support for this claim "Extension Toxicology Network. 1993. http://extonet.orst.edu/pips/24-D.htm (citing Howard, Philip H. Handbook of Environmental Fate and Exposure Data for Organic Chemicals. Lewis Publishers Chelsea, Michigan.)." The Extension Toxicology Network's Pesticide Information Profile on 2,4-D, prepared almost 14 years ago in 1996, does not state that 2,4-D is found "in about half of all surface water samples across the U.S." Nor does the parenthetical Howard reference so state. Rather, at page 151 the Howard

reference states that "[i]n a U.S. National Surface Water Monitoring Program conducted during 1976-89, 2,4-D was detected in 1.6% of surface waters at a maximum concentration of 1.9 ppb". The Maximum Contaminant Level (MCL) and Maximum Contaminant Level Goal (MCLG) for 2,4-D in drinking water under the Safe Drinking Water Act are both 70 ppb; 1.9 ppb is less than 1/30th of the MCL and MCLG. In short, NRDC's claim is wrong and unsupported. Additionally, EPA published in FR Notice Vol. 59, page 15531, March 29, 2010, results of sampling of 33,187 water systems in the US. This sampling showed no detections of 2,4-D at 0.070 mg/L, nondetects at a lower level (0.040 mg/L) nor at the limit of detection. These water systems served an estimated population of 187,451,200 people.

EPA's 2005 2,4-D Reregistration Eligibility Decision ("2,4-D RED") (USEPA 2005) reviewed the available surface water and groundwater monitoring data for 2,4-D in the Revised Drinking Water Assessment document for the 2,4-D Reregistration Eligibility Decision Health Effects Division Chapter. The EPA-reviewed databases consisted of the USGS National Water-Quality Assessment Program (NAWQA), the USEPA STOrage and RETrieval System (STORET), and USGS Pilot Reservoir Monitoring Study. The maximum 2,4-D concentrations observed were 58 ppb in surface water and 14.8 ppb in groundwater. In the NAWQA database going back to 1991 2,4-D was found in only 0.5% of the 4340 groundwater samples above the LOQ of 0.035 ppb. In the STORET database 2,4-D was only detected above the LOQ in 2.8% of the 21,232 samples.

The EPA developed the National Drinking Water Contaminant Occurrence Database (NCOD) to address requirements of the 1996 amendments to the Safe Drinking Water Act. The database stores data from Public Water System analytical results on finished drinking water. The database shows that 2,4-D was detected in only 0.6 % of the 12,654 samples reported.

EPA developed Estimated Exposure Concentrations (EEC) for drinking water from both modeling and monitoring data in the 2,4-D RED and concluded that these concentrations did not exceed Agency's Level of Concern.

NRDC cited studies conducted at doses not relevant to human risk assessment

A number of the studies cited by NRDC used non-representative doses not relevant for human risk assessment based on the saturation kinetics for 2,4-D.

In designing a toxicity study for the evaluation of human risk, it is critical to select an exposure regimen that is representative of the human pharmacokinetics within the exposure range under consideration. For example, Saghir and co-workers demonstrated saturation of renal clearance and distinctly non-linear pharmacokinetics in male rats fed 100 mg/kg/day 2,4-D for 28 days (Saghir *et al*., 2006). Use of rodent study doses that do not exceed the KMD is an important consideration in the evaluation of potential human health risks of 2,4-D exposure, in that both rodents and humans share the same renal clearance mechanism for 2,4-D (Timchalk, 2004). Rodent toxicity observed above such non-linear pharmacokinetic dose levels, *i.e*., above the KMD, is not relevant to human risk in that such doses result in disproportionately elevated systemic doses. Importantly, the dog, because of distinctly different renal clearance mechanisms relative to rats and humans and resulting dramatically different pharmacokinetic behavior relative to rats (van Ravenzwaay *et al*., 2003; Timchalk, 2004), is not an appropriate species for evaluation of human health risks for 2,4-D.

To derive an appropriate dosing regimen, the recent extended F1-extended one generation reproductive toxicity study (Marty *et al*. 2010) derived a kinetically-derived maximum dose, or KMD, which takes into account the pharmacokinetics of 2,4-D in the test species, including during pregnancy and lactation. The dietary range-finding and toxicokinetics study and the titration study conducted prior to the Marty *et al.* 2010 study more precisely identified the doses at which renal saturation resulted in non-linear pharmacokinetic performance as approximately 25 mg/kg/day for female rats and 65 mg/kg/day for males (Saghir *et al*., 2008a). These dose levels can be regarded as the KMD for 2,4-D administered in the diet to rats. Studies administering doses significantly above the KMD are not representative of human exposure or predictive of human risk.

2,4-D IS NOT AN ENDOCRINE DISRUPTOR

NRDC claims that 2,4-D has "extensive hormone disrupting activity" and that these effects "may occur at low doses". In fact, the scientific record indicates 2,4-D has a very low endocrine disrupting potential. Endocrine effects in animal toxicity studies attributed to 2,4-D are restricted to unrealistically high doses that exceed saturation of renal clearance of 2,4-D in rodents and thus are of limited value to human or animal risk assessment. In addition, the doses to which animals are exposed in studies are all substantially greater than possible human exposures as demonstrated by extensive occupational and general population biomonitoring studies (see Exposure Section below). Although NRDC claims that EPA "illogically ignores the existing data," EPA in March, 2007 issued a data call-in for a two-generation reproduction study (Guideline No. 870.3800) that specifically required examination of thyroid, gonadal, reproductive and other endocrine-sensitive endpoints. In addition, EPA also requested an assessment of both developmental neurotoxicity and immunotoxicity potential. Studies assessing these endpoints have been completed.

As noted above, EPA is currently reviewing an F1-extended one generation study (Marty *et al*., 2010) which addressed multiple endocrine-related endpoints and did not find exposure- related adverse endocrine effects. The endpoints evaluated included the following:

- Estrogen sensitive endpoints: Estrogen sensitive endpoints evaluated included estrous cyclicity assessment (in both parental females and F1 offspring), time to vaginal opening (including notation of retained vaginal threads), anogenital distance, quantitative ovarian follicle count, reproductive organ weight evaluations and detailed histopathology of female reproductive organs across multiple life stages. No effects suggesting 2,4-D was estrogenic or anti-estrogenic were evident in these studies. These multiple endpoints are sensitive to estrogenic and/or anti-estrogenic MOA, and showed no evidence of exposure-related effects.

- Thyroid effects: The F1-extended one-generation study evaluated thyroid hormones (T3, T4 and TSH), thyroid weight and/or thyroid histopathology across life stages, and also evaluated motor activity, auditory startle and brain morphometric data (which have been reported to be sensitive to thyroid disruption) in the DNT component of the study. There were no exposure-related findings in adult male rats. Exposure-related findings in adult females in the F1-extended one-generation study were limited to findings in late gestation of high dose non-statistically significant decreases in T3 and T4 and increased TSH, associated with adaptive changes (depleted colloid) in the thyroid follicles of GD 17 females at a dose which clearly exceeded the threshold for saturation of renal clearance. There was no indication of thyroid proliferative changes. Statistically significant changes were seen in T4 in high dose male weanlings; however there were no associated changes in TSH or in thyroid histopathology and the findings were not considered adverse. No robust effects on auditory startle or statistically significant brain morphometric changes were seen in the DNT component of the extended one-generation reproductive toxicity study, findings that otherwise might be expected as sequelae to any marked thyroid hormone deficiency. Myelin deposition was also characterized at PND 22 and 60, and no exposure-related changes were observed. Overall, there are no findings suggesting an effect on the thyroid at doses below the saturation threshold for renal clearance.
- Androgen-sensitive effects: Androgen or anti-androgen sensitive parameters evaluated in this study included many sensitive to anti-androgenicity or androgenicity, including balano-preputial separation, anogenital distance, quantitative F1 nipple retention (considered particularly responsive to anti-androgenicity), spermiology, organ weights and detailed histopathological evaluations of male reproductive organs. No exposure-related effects were seen for those parameters.

Note that in addition to the findings in the F1-extended one generation reproduction study, findings from other chronic studies of 2,4-D support that 2,4-D has limited potential for endocrine activity. For example, there were no exposure-related increases in

the incidence of estrogen-responsive mammary tumors in either the rat or female mouse oncogenicity studies.

2,4-D DOES NOT SHOW DEVELOPMENTAL IMMUNOTOXICITY

2,4-D had no effect on humoral immune function in males (all dose levels) or females exposed to ≤300 ppm as measured by antibody plaque forming cell (AFC) responses on PND 70-74. High-dose females had non-significant decreases in AFC/spleen and AFC/106 splenocytes, which appeared to be due to temporal variability over the 4-day evaluation span. This occurred at a dose (600 ppm), which demonstrated non-linear toxicokinetics. For innate cellular immunity, the natural killer cell (NK) assay (PND 87-93) measured target cell cytotoxicity using fluorescent labeled YAC-1 cells (targets) plated with spleen cells (effectors) at E:T ratios from 50:1 – 800:1. The NK assay showed no effects from 2,4-D exposure; linear cytotoxicity with increasing E:T cell ratios was identical across all doses. Thus, there was no evidence of DIT effects related to 2,4-D exposure (Marty *et al*., 2010).

THERE IS NOT LIKELY TO BE A NEED FOR ADDITIONAL UNCERTAINTY OR SAFETY FACTORS BASED ON THE RESULTS OF THE F1-EXTENDED ONE GENERATION REPRODUCTIVE TOXICITY STUDY

Prior to completion of the F1-extended one generation study EPA applied a 10X database uncertainty factor (for a total 1000X) to account for the lack of the required reproduction and developmental neurotoxicity studies. Once EPA has reviewed the findings of the Marty *et al*. (2010) study it is considered likely that the data will be found to be sufficient to remove this uncertainty factor, because this study provides state-of-the art scientific data to address these concerns. As described previously, this study included a comprehensive analysis of endocrine and DNT endpoints (as well as systemic toxicity and developmental immunotoxicity) and did not identify exposure- related adverse effects, except previously known renal toxicity. Importantly, the F1-extended one generation study provided no support for a lower NOAEL than established in prior rat

studies, and provided no evidence of unique sensitivity to the developing young. Thus, the new data do not indicate a need to modify the animal NOAELs and LOAELs previously established from existing studies (EPA response to public comments OPP-2004-0167-0090 16-Dec-04) with either an FQPA Safety Factor (to account for potential sensitivity to the developing young) or with any other database uncertainty factor. The 2,4-D Task Force is relying on the F1-extended one generation study in lieu of conducting selected Tier 1 endocrine screening studies recently required by EPA; however, the Agency on several occasions has rejected the position that "data gathered under the Endocrine Disruptor Screening Program ("EDSP") is a prerequisite to a safety determination under FFDCA section 408." (US Federal Register 2007a at 68662, 68676) (denying NRDC petition to cancel DDVP, Dec. 5, 2007); (US Federal Register 2006 at 43906, 43919-43921) (denying states petition to cancel certain pesticides).

Thus, the results of the data call-in studies are unlikely to alter the RED 2,4-D risk assessments by a factor greater than those already imposed by the 10X intra and interspecies uncertainty factors. The 2,4-D Task Force believes this study provides state-of-art toxicological evidence to support either eliminating or greatly reducing application of the full currently applied 10X data gap uncertainty factor.

NRDC asserts that EPA "relies on the hollow excuse that a formal screening program does not yet exist to avoid examining potential endocrine effects". This is incorrect. EPA had imposed an extra 10X data gap uncertainty factor that reasonably accounts for the lack of a state-of-art reproductive assessment which includes a more detailed assessment of endocrine endpoints. Moreover, the F1-extended one generation study recently submitted to EPA, and based on a study design approved by EPA which, as detailed above, included multiple endocrine-sensitive endpoints (*e.g.*, estrous cycle activity, ano-genital distance, and sperm parameters) and this study supplants the requirement for any mammalian toxicity information elements gleaned from the required Endocrine Disrupting Screening Program Tier 1 studies or proposed Tier 2 studies.

Contrary to NRDC's assertions, the estrogenic properties of 2,4-D have been extensively examined in both mammalian and non-mammalian *in vitro* and *in vivo* studies. These studies, discussed below, do not support NRDC's claim that 2,4-D is a "potent" endocrine disruptor in any species. It is very unlikely that 2,4-D is an endocrine disruptor or stressor given the extremely low human exposures to the herbicide.

NRDC cites Xie *et al.* (2005) as reporting that 2,4-D has a "relatively potent estrogenic effects in fish." However, the authors of the study refer to their findings as showing elevated estrogenic activity, as measured by elevation of plasma vitellogenin, under conditions of a "worst-case scenario" exposure (1.64 mg/L 2,4-D). The NOEC and LOEC values for this response were reported as 0.0164 mg/L and 0.164 mg/L, respectively. As noted in the comments above in response to the NRDC claim that 2,4-D was detected in 50% of sampled waters, a U.S. National Surface Water Monitoring program reported finding 2,4-D in only 1.6% of samples, and then only at a maximum concentration of 1.9 ppb (0.0019 mg/L). Thus repeated field monitoring indicates a large separation between the responses in this laboratory study and 2,4-D concentrations reported in surface water.

Interpretation of Xie *et al.* is further confounded by several other observations. The reported vitellogenin responses appear to be variable and inconsistent across experimental parameters such as dose and presence of surfactants. Such variation confounds clear identification of NOEC and LOEC values reported in this study. Xie *et al.* also noted that a combined treatment with 1.64 mg/L 2,4-D and 1.46 mg/L of the surfactant R-11 resulted in approximately 16-18 ng/mg vitellogenin expression. A subsequent experiment using a similar treatment period and 2,4-D concentration (1.64 mg/L) but a *lower* concentration of R-11 (0.89 mg/L) resulted in much *higher* vitellogenin expression (approximately 50 ng/mg). In addition, the mechanism of the high-concentration induced vitellogenin response remains unclear. Numerous studies have demonstrated that 2,4-D does not bind to or activate mammalian or fish estrogen receptors (Table 1). Further, a major photolysis/hydrolysis environmental metabolite of 2,4-D, 2,4-dichorophenol, also exhibits no or extremely weak *in vitro* estrogen receptor

activity (Korner *et al*., 1998; Nishihara *et al*., 2000).

Thus, Xie *et al*. (2008) does not provide evidence that 2,4-D has "potent" estrogenic activity, and the variable nature of the reported responses requires further investigation and replication before inferring either fish or mammalian environmental health risks.

Table 1 lists those assays indicating that 2,4-D is negative for estrogenicity.

Table 1. In vitro assays indicating 2,4-D is negative for estrogenicity	
Nishihara *et al*., 2000. J. Health Sci. 46(4):282-298	Yeast two-hybrid screen negative[1]: negative
Blair *et al*., 2000. Tox. Sci. 54:138-153	Rat estrogen receptor binding: negative
Petit *et al*., 1997. J. Mol. Endocrinol 19:321-335	YES[2]: negative Trout estrogen receptor binding: negative Trout hepatocyte vitellogenin: negative
Hwang, 2002. Han-guk Yanksik Hakhoechi 15:31-37	Trout hepatocyte vitellogenin: negative Estrogen receptor binding: equivocal
Hurst and Sheahan, 2003. Sci. Tot. Env. 301:87-96	Human YES: negative
Jung *et al*., 2004. Life Sci 74:3065-3074	YES: negative
Kojima *et al*., 2003. EHP 112:524-531	Human estrogen receptor-alpha, estrogen receptor-beta transactivation: negative
Vonier *et al.,* 1996. EHP 104(12):1318-1323	Alligator estrogen receptor binding: negative
Soto *et al*., 1995. EHP suppl 7: 113-122	MCF7 Cell proliferation: negative
Jungbauer and Beck, 2002. J. Chromatog. B. 777:167-178	Human YES: negative
Lin and Garry, 2000. J. Tox. Env. Health A 60:423-439	YES for 2,4-D active ingredient: negative YES formulated material (which may contain estrogenic surfactants): positive
Judson, *et al*., EHP 2009 and supplementary materials (Report from ToxCast™ assays)	Cell free assays: ∞ Bovine estrogen receptor from uterine membrane (bER): negative; ∞ Human estrogen receptor from MCF-7 cells (hER) negative; Cell-based assays: ∞ Agonist activity at the human estrogen receptor α: negative ∞ Antagonist activity at the human estrogen receptor α: negative; Multiplex transcription reporter gene assay: ∞ Human androgen receptor: negative ∞ Human estrogen receptor-α: negative

Table 1. In vitro assays indicating 2,4-D is negative for estrogenicity	
	∞ Human estrogen-related receptor-α: negative ∞ Human estrogen-related receptor-γ: negative
[1.] Indicated incorrectly by Xie *et al*. (2005) as "estrogenic at a concentration of approximately 0.2 g/L". [2.] YES = Yeast Estrogen Screen or yeast two-hybrid screen	

Evidence of 2,4-D estrogenic activity has not been demonstrated in several whole organism toxicity tests. As discussed above, the F1-extended reproductive toxicity study by Marty *et al*. (2010) did not identify estrogenic effects in analyses of multiple endpoints that are sensitive to estrogenic and/or anti-estrogenic modes of action (MOA), and showed no evidence of exposure-related effects.

In ovo exposure of alligator eggs to a range of 2,4-D concentrations had no effect in the hatchlings to the estrogen-sensitive endpoints of 1) production of females at male-determinant egg incubation temperatures; 2) gonadal and reproductive histology (females: epithelial cell in Mullerian duct and medullary regression of ovary; males: sex-cord diameter); and 3) hepatic or gonad-adrenal-mesonephros (GAM) aromatase activity (Spiteri *et al*., 1999).

In a series of GLP subchronic 90-day dietary toxicity studies in rats, 2,4-D acid and its commercial salt and ester derivatives produced only minimal or no responses in estrogen and/or androgen sensitive tissues, and when observed, were restricted to high doses which exceeded either or both the maximum tolerated dose ("MTD") and renal clearance capacity (Charles *et al*., 1996). In a GLP developmental toxicity study of 2,4-D and its salt and ester derivatives in rats and rabbits, offspring effects were again limited to high maternal doses exceeding renal clearance saturation and were not characteristic of endocrine-directed targets (Charles *et al*., 2001). Finally, reproductive studies conducted with either a mixture of 2,4-D and picloram (Oakes *et al*., 2002) or with 2,4-D alone

(study submitted to support re-registration of 2,4-D, Rodwell and Brown, 1986) resulted in high-dose only effects that were not characteristic of endocrine-mediated responses (*i.e*., no effects on reproductive tract malformations, retained nipples in males, pre-coital length, fertility, histological alterations in accessory sex glands, epididymides, testes, ovaries and uteri). Although the Rodwell and Brown study reflected guideline protocol design when it was conducted in 1985, the Task Force's recently submitted F1-extended one-generation reproduction study which was completed to satisfy EPA's data call-in used a state-of-art assessment methodology based on a protocol approved by EPA including endocrine-sensitive endpoints and as such further supplements and updates the reproductive toxicity dataset for 2,4-D. The study included comprehensive evaluation for reproductive endpoints including included many sensitive to anti-androgenicity or androgenicity, including balano-preputial separation, anogenital distance, quantitative F1 nipple retention, spermiology, organ weights and detailed histopathological evaluations of male reproductive organs and found no exposure-related effects (Marty *et al*., 2010). As noted above this study also evaluated endpoints sensitive to estrogenic or thyroid effects.

Further, evaluations of oncogenicity have not found exposure-related increases or decreases in endocrine-sensitive tumors (Jeffries 1995, MRID 43612001).

NRDC concerns regarding potential human health implications of 2,4-D effects on thyroid hormone alterations also are not supported by the data. In the subchronic rat studies cited by NRDC and described above (Charles *et al*., 1996), decreased serum levels of T4 and/or T3 were noted only at high doses that exceeded the KMD based on renal clearance, and minor histological effects in the thyroid were also seen only at a dose which exceeded the MTD (300 mg/kg/day). The ewe study cited by NRDC (Rawlings *et al*, 1998) is too limited in its design to provide useful information regarding potential thyroid effects of 2,4-D. The study used only a single 2,4-D dose and a limited number of animals per treatment group. While the study noted a small but significant decrease in serum T4, this observation was not accompanied by any change in thyroid histopathology and TSH was not measured.

Marty *et al*., 2010 evaluated thyroid endpoints including thyroid hormones (T3, T4, and TSH), thyroid weights and/or histopathology across multiple life-stages, including pregnant females, pups at PND 4 and PND 21 and non-pregnant adults exposed for greater than 10 weeks. The majority of these endpoints showed no evidence of exposure-related effects. There was a statistically significant decrease in T3 only (19%) at the 300 ppm dose group in PND 22 male pups ; however, this decrease exhibited a poor dose-response relationship as 600 ppm T3 levels were decreased only 13%. This finding is attributed to variation in circulating T3 levels and is not considered to represent an exposure-related effect. T4 levels were significantly decreased (28%) in the 600 ppm male weanlings (PND 22), which was a dose exceeding the KMD. However, there was no corresponding increase in TSH levels indicative of a thyroid response to decreased thyroid hormone, and no histopathological evidence of any changes, adverse or otherwise, in thyroid tissue. Therefore the findings in pups are not considered to represent adverse effects. There was also a pattern of slight thyroid effects in pregnant female rats at a dose exceeding the KMD. These findings included a slight non-statistically significant decrease in T4 and non-statistically significant increase in TSH, evident histopathologically only as slight colloid depletion in 3/12 female rats. Colloid depletion is considered an adaptive response to changes in circulating thyroid hormone levels and does not represent an adverse effect. The thyroid effect [in female rats or in male and female rats] has been characterized across life stages, the mechanism is understood, and a clear NOAEL determined. Moreover, there were no exposure-related alterations in any DNT-related endpoints such as brain morphometrics, which support the conclusion of no biologically relevant changes in thyroid function.

NRDC also describes a series of other studies it contends provides evidence of 2,4-D endocrine activity. None, however, provide compelling evidence of potential environmental or human endocrine health concerns. Specific comments on these studies are as follows:

1. Liu *et al.* (1996) found no significant effects of 2,4-D in hCG-stimulated release of testosterone in rat Leydig cells cultures. Although NRDC states 2,4-D

produced a significant increase in estrogen release from these cells, it neglects to mention the *minimal* effect concentration for this effect was 500 µM. This concentration translates to approximately 110 µg/ml, and is substantially greater than the maximum 2,4-D plasma concentration reported in rats treated with 100 mg/kg/day in the diet (63 µg/ml; Saghir *et al*., 2006). Given that 100 mg/kg 2,4-D is well above saturation of renal clearance, *in vitro* findings in this concentration range are not indicative of an endocrine risk.

2. The *in vitro* mechanistic study of Kim and co-workers (2005) is based on their earlier report that 2,4-D increased prostate weight in rats treated with 50 mg/kg/day by oral gavage (Kim *et al.,* 2002). However, the effect on prostate weight occurred at a dose and route of administration (oral gavage) known to be above the threshold for saturation of renal clearance. Thus, the observation underpinning the mechanistic *in vitro* study has not yet been examined in standardized guideline toxicity studies and the use of the high dose limits the usefulness of this *in vitro* study. The findings in Kim *et al*. contradict those of Fang *et al*. (2003), which used recombinant rat AR protein and tested a wider range of test compound concentrations (4.28 x 10-9 to 4.28 x 10-4 M). The results also contradict the results of the ToxCast™ program screening both in cell-free and cell-based assays, which showed no interaction with the androgen receptor (Judson *et al*., 2009). Finally, although 2,4-D and DCP were found to bind the human AR in the study of Kim *et al*. (2005), these compounds alone did not induce proliferation of an androgen-response prostate cancer cell line or activate the human AR, as demonstrated in the receptor transactivation assays. The Kim *et al*. study is considered limited and the findings are difficult to interpret. The effects of dietary 2,4-D treatment on prostate weight and pathology was examined in the F1- extended one-generation reproduction study and no exposure-related effects were found within relevant dose levels (Marty *et al*., 2010).

The effects of 2,4-D on prolactin, progesterone and estrous cycle alterations described in Duffard *et al*. (1995) were at a dose level well above saturation of renal clearance and thus are not relevant for human risk assessment. The findings in the Duffard *et al*. (1995) study were not replicated in the F1-extended one generation study, which had much better characterized exposures, and comprehensively evaluated estrogenic parameters, including estrous cyclicity, and did not identify treatment related effects.

3. Lerda and Rizzi (1991) does not support the contention that 2,4-D causes endocrine effects in farmworkers because of several reporting and methodological weaknesses. Many study-specific details were not reported including background of controls, number of participants excluded due to spermatogenesis-affecting health conditions, method used in "consideration" of external factors, detection limit for 2,4-D in urine, time period of urine collection, and ranges of sperm parameters and 2,4-D urine levels evaluated (only means provided in the report). In addition, the selection of controls was inappropriate as comparison was to workers in the field exposed to 2,4-D, but the controls were not agricultural workers doing similar field work. Field work involves exposure to other confounding factors (*e.g*., increased temperature, dusts, allergens) that likely alter sperm parameters.

4. The study of Garry and co-workers (1996) assessing potential associations of *herbicide* use and birth defect outcomes in an agricultural region of Minnesota was later described by this same research team as "a first effort" that suffered from a "limited reporting timeframe" (Garry *et al*., 2002). In the latter report, the authors concluded that a more detailed cross-sectional analysis of this area showed no statistically significant correlations between 2,4-D use and excess adverse birth or neurodevelopmental effects.

In summary, the Task Force respectfully submits that the weight of evidence does not support NRDC's claim that 2,4-D "has been shown to have extensive hormone-disrupting activity." The Task Force's recently completed F1-extended one generation reproduction study supplements existing *in vivo* and *in vitro* studies that demonstrate that 2,4-D does not affect the endocrine system at doses below a KMD. As described previously, this study included a comprehensive analysis of endocrine and DNT endpoints and did not identify exposure-related adverse effects. The non-GLP studies cited by NRDC suffer from significant methodological or reporting limitations, or they administered doses far in excess of those to which humans would ever be exposed (and far above the KMD). Finally, EPA has repeatedly stated that data gathered under the FQPA EDSP is not a prerequisite to a determination that a pesticide chemical residue meets the safety standards of FFDCA § 408.

NEURODEVELOPMENTAL TOXICITY

NRDC claims that "2,4-D is neurotoxic." It supports this contention by citing findings in several studies in the published literature. NRDC ignores, however, significant problems with the design, route of administration, and in some cases even the test material in the studies cited, making the findings in these studies an inappropriate basis for risk assessment or regulatory decision-making on 2,4-D. For example, NRDC cited a zebrafish embryo study by Ton *et al*. (2006) as evidence of developmental neurotoxicity. This study did not assess developmental neurotoxicity but rather was a method development exercise designed to create a screening system for potential developmental neurotoxicity. There were no negative controls in the Ton *et al*. study. In addition, the lowest concentrations affecting some of the endpoints cited by NRDC, such as disrupted motor neuron growth, occurred at concentrations that exceeded the LC50 for zebrafish (despite NRDC's claim that the exposure was "sub-lethal"); therefore, the reported endpoints do support the contention that they are indicators of developmental neurotoxicity. As an attempt to create a screening assay, its protocol clearly lacked the sensitivity and specificity required to generate data that would enable a regulator to

assess whether 2,4-D affects the developmental neurotoxicity of fish (and even less to humans).

NRDC next cite the same Ton *et al*. 2006 zebrafish paper to support their claim that 2,4-D is associated with developmental neurotoxicity in mammals, "including decreased motor activity and Parkinson's-like tremors." (NRDC Pet. at 6.) The problem is Ton *et al*. never tested mammals. In the introduction to their fish study, Ton *et al*. claim an association between 2,4-D and decreased motor activity and tremors in mammals but Ton's support for their claim, in turn, is a news and view commentary by Giasson and Lee, 2000 who discuss using the pyrethroid insecticide and pesticide rotenone as a model for Parkinson's disease. Giasson and Lee never even mentioned 2,4-D.

NRDC then cites a collection of rat studies (NRDC footnotes 34 - 38) all of which administered high dose levels that have been shown to overwhelm metabolic clearance. Specifically, these studies all tested doses of 2,4-D at 70-100 mg/kg ip or sc, which exceeds the threshold for renal clearance of 2,4-D (established in oral [gavage and dietary] studies: van Ravenzwaay *et al*., 2003; Saghir *et al*., 2006). Further, a recent range-finding study of 2,4-D in CD® rats (Saghir *et al*., 2008a) showed overt maternal and pup toxicity in the same dose range, reflected in severely decreased maternal feed consumption during lactation and marked pup weight gain decreases and increased mortality (at a 100 mg/kg bw/day dietary dose). This study also evaluated toxicokinetic parameters and more precisely identified the doses at which renal saturation resulted in non-linear pharmacokinetic performance as approximately 25 mg/kg/day for female rats and 65 mg/kg/day for males. Findings from these studies were presented at the 2009 SOT and the range-finding and titration studies (Saghir et al., 2008a, 2008b) were reviewed by EPA and accepted as the basis for dose selection for the definitive F1-extended one-generation study.

Among the mammalian studies cited by NRDC was a study relating to neurotoxicity or developmental neurotoxicity that did not appear to test overwhelmingly high doses (Bortolozzi *et al*. 2001). This study, however, was an experimental test where 2,4-D was

directly administered into the rat's brain. This route is not relevant to human or mammalian risk assessment and it is unfortunate that NRDC relies on such a study.

Other mammalian studies cited by NRDC are summarized below:

1. NRDC cites Evangelista de Duffard *et al*. (1995) in support of the claim that 2,4-D causes "delays in brain development and abnormal behavior patterns, including apathy, decreased social interactions, repetitive movements, tremor and immobility." (NRDC Petition at 7). First, the study does not assess brain development. Second, the findings in this study cannot be attributed to 2,4-D alone. Indeed, the study is entitled "Altered behavioral responses in [2,4-D] treated and amphetamine challenged rats" designed to be a pharmacological challenge study in adult animals (thus not assessing brain development). The effects were a result of acute toxicity of intraperitoneal amphetamine, spiperone and/or haloperidol (all of which are directly neurotoxic), possibly enhanced by a prior high (50 or 100 mg/kg) acute intraperitoneal dose of 2,4-D. The authors clearly state in the abstract, "all behaviors were not seen in the 2,4-D treated rats." Finally, the studies by this group of researchers are high dose evaluations and many suffer from methodological deficiencies, particularly in dose formulation and analysis, which make interpretation and reliability questionable.

The observations NRDC reports of "decreased social interaction and apathy" were made in animals given combined 2,4-D and amphetamine doses that caused lethality, which indicates that these behaviors may be non-specific effects rather than any selective neurological effect. The data supporting effects on these particular "social" behaviors is especially weak. There is no description in the methods section of how "social interaction" or "social apathy" was evaluated, and the reporting of these results is not sufficiently rigorous to allow any conclusions to be made.

2. NRDC cites Bortolozzi *et al*. (2001) to support its claim that "[r]odent 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."

(NRDC Pet. at 7). The route of administration in Bortolozzi *et al*. was direct injection of 2,4-D in DMSO into specific brain areas of the rat. Bortolozzi *et al*. indicated that the estimated 2,4-D concentrations in each brain area were 2-4 mM, which the author defined as 40 to 100-fold the concentration in brain after systemic treatment. This study is not a developmental neurotoxicity study but a very high dose adult toxicity study using a route of exposure that has no relevancy to human exposures.

3. NRDC concludes by citing three papers from the same research group (Rosso *et al*., 2000; Duffard *et al*., 1996 and Konjuh *et al*., 2008) to allege that "this herbicide specifically appears to impair normal deposition of myelin in the developing brain." (NRDC Pet. at 7). These were the only evaluations of actual developmental neurotoxicity in mammals among the studies cited by NRDC. These studies, however, were conducted at excessively high doses of 70 and/or 100 mg/kg/day 2,4-D either through subcutaneous or intraperitoneal injection, which are dose levels that would be expected to exceed the threshold level for saturating renal clearance of 2,4-D. No effects on myelin in the developing brain was found in Marty *et al*. (2010), which evaluated myelin deposition in both weanlings and F1 adults using special staining. The two earlier studies (Duffard *et al*., 1996; Rosso *et al*., 2000) were methodologically flawed, because the litter was not used as the unit for statistical analyses, which is critical to a developmental neurotoxicity evaluation to avoid bias due to litter effects. The reporting of the histopathological evaluation of these two studies is limited, with no sample size reported, and insufficient reporting of methods and results to allow a conclusion that unbiased objective measurements were made using a sufficient sample size. The authors themselves admit that their findings could be due in part to undernutrition and resulting weakness caused by maternal toxicity instead of a specific 2,4-D effect on the developing nervous study.

The hypothesis that the effects reported by Duffard *et al*. and by Rosso *et al*. may have been due to undernutrition was tested in a more recent study by this group (Konjuh *et al*., 2008), in which the experimental design was somewhat improved compared to the earlier

studies noted above. In the Konjuh *et al*. study "undernutrition" was achieved by retaining 14 pups per litter, while "normal" nutritional status was achieved by culling to the more standard litter size of 8 pups litter. Although Konjuh *et al*. (2008) report some differences in myelin parameters between undernourished pups and those exposed intraperitoneally to 2,4-D, the general pattern of 9 of the 12 myelination parameters evaluated actually appeared similar between undernourished and 2,4-D well-nourished pups This evaluation is considered too limited to be more than a hypothesis generating study because there was only one exposed dose group (70 mg/kg/day), the sample size was too small (single pups from each of 4-5 litters), and no corrections were made for multiple statistical comparisons.

In summary, the papers cited by NRDC do not provide credible or substantive evidence that 2,4-D causes developmental neurotoxicity at exposure levels or routes of administration relevant to humans. In contrast, as discussed in more detail in the Exposure section below, exposure levels as reflected in biomonitoring are very low in families of farm workers based on measures during a period of high 2,4-D use (Alexander *et al*., 2007) and in an EPA-conducted biomonitoring study of adult and residents from randomly selected households in North Carolina and Ohio (Morgan *et al*., 2004, 2008). Comparison of these measured biomonitoring results with internal dose levels estimated to be associated with the EPA RfD for 2,4-D (termed biomonitoring equivalents), suggests a considerable safety margin between existing exposure levels and levels identified by EPA as safe and the authors concluded:

> " Biomonitoring data from these studies indicate that current exposures to 2,4-D are below applicable exposure guidance values." (Aylward and Hays, 2010).

As noted, in response to EPA Data-Call-In requirements, the 2,4-D Task Force sponsored a F1-extended one-generation reproduction study in rats of 2,4-D in the diet which included assessment of developmental neurotoxicity endpoints and found no exposure-related DNT effects, or effects on myelin deposition in the developing brain.

MUTAGENICITY AND GENOTOXICITY

NRDC alleges that EPA "disregarded a number of studies that highlight the mutagenicity and genotoxicity of 2,4-D." (NRDC Pet. at 7). NRDC claims EPA only considered data on the "pure substance" or active ingredient. The NRDC petition then goes on to cite various studies as supportive of its views.

In fact, the test data submitted in support of 2,4-D reregistration evaluated 2,4-D acid plus eight different 2,4-D derivatives, *e.g.*, 2,4-D dimethylamine salt and 2,4-D 2-ethylhexyl ester. Each of the derivatives was subjected to a battery of mutagenicity tests leading to a total of 28 studies submitted for reregistration, **all of which were negative** (non-mutagenic). NRDC omits mention of any of these studies or the three published papers reviewing them. The two papers by Charles *et al*. (1999a; 1999b) and a paper by Gollapudi *et al.* (1999) state, "There was no evidence of genotoxicity for potential for 2,4-D acid, or any of its derivatives in these assays." Importantly, the 28 studies submitted for reregistration met stringent regulatory testing guidelines and complied with EPA's Good Laboratory Practices (GLP) Standard as required by the Code of Federal Regulations 40CFR, Part 160.

Furthermore, EPA acknowledges that some positive mutagenicity studies occur in the published literature on 2,4-D. However, the weight of the evidence overwhelmingly supports a conclusion of minimal or no concern for mammalian mutagenicity from 2,4-D exposure. It must be remembered that the 1988 EPA Registration Standard required new mutagenicity studies for all 2,4-D forms because the existing studies did not meet current testing guidelines or GLP requirements. A strong and substantial set of well documented studies is now available to support a diligent EPA risk assessment and overall risk management conclusion.

A 2002 review published by Garabrant and Philbert also provides another weight of evidence type conclusion: "Despite several thorough *in vitro* and *in vivo* animal studies, no experimental evidence exists supporting the theory that 2,4-D or any of its salts and esters damages DNA under physiologic conditions." (Garabrant and Philbert, 2002).

Several inherent characteristics of 2,4-D suggest that there is a very low potential for it to cause mutagenic effects:

- The half-life of 2,4-D in humans is less than 12 hours.
- 2,4-D does not metabolize or transform.
- 2,4-D is excreted unchanged.
- 2,4-D does not accumulate.

Among the studies cited by NRDC, Madrigal-Bujaidar *et al*. (2001) reported an increased frequency of sister chromatid exchange in bone marrow and spermatogonial cells of mice exposed *in vivo* to 100 mg/kg of 2,4-D. The mid and high doses of 100 and 200 mg/kg in this study are markedly greater than the renal clearance threshold for 2,4-D in the rodent and are massively larger than realistic exposures for humans. However, even at these very high doses, effects were weak. The study's low dose of 50 mg/kg having no reported effect is also at or above the renal clearance threshold and above realistic exposure levels (Madrigal-Bujaidar *et al*., 2001).

Holland *et al*. evaluated *in vivo* and *in vitro* 2,4-D exposures and showed a 12-15% increase in the replicative index, but no biomarker of genotoxicity was found (Holland *et al.,* 2002). EPA reviewed this study and cited it in the 2,4-D RED. It is considered negative for mutagenicity.

Figgs *et al.* conducted a longitudinal study of pesticide applicators and found that urine concentrations of 2,4-D increased logarithmically as spraying time increased (Figgs *et al.,* 2000). Most parameters evaluated showed no effect, but the authors hypothesized that a finding of increasing lymphocyte replicative index could be attributed to the dose of 2,4-D. It is clear that this study had limitations such as the small number of subjects and an untested design, to name a few. The authors self-described the study using the terms "preliminary", "pilot investigation", and "the first to show a relationship". Going on, they said, "The link between 2,4-D function and lymphocyte replicative index is unclear." And, "Whatever the mechanism, our reports and other recent investigations do not support a genotoxic pathway."

In the science of toxicology, substantial evidence of mutagenicity is usually considered to suggest a potential for carcinogenicity. For this reason, lifetime animal bioassays are the gold standard for more definitive determinations. With 2,4-D having a very rich toxicological database, the hallmarks of classical mutagenicity and carcinogenicity can be considered with rare conviction. Not only are there 28 high quality mutagenicity studies, but four modern carcinogenicity studies have been conducted on 2,4-D including two studies in the rat and two studies in the mouse (USEPA Memorandum. 2004b). All of these GLP studies were evaluated by EPA and reported in the 2,4-D RED (USEPA 2005). The weight of evidence across the rodent bioassays is that 2,4-D is not carcinogenic, which is corroborative of the negative mutagenic evidence. The occasional contradictory studies in the mutagenicity database ultimately point to the plentiful rodent bioassay studies which show an absence of carcinogenic potential of 2,4-D.

EXPOSURE

Exposure through maternal milk

NRDC claims that EPA's aggregate exposure was inadequate because the Agency failed to consider that ''[t]here is evidence of exposure to 2,4-D through maternal milk" citing the Sturtz *et al.* (2000) study finding that 2,4-D is excreted in maternal milk in rats, and NRDC thereby inferred potentially significant exposures to the nursing neonates. NRDC claims 2,4-D residues were found in the stomach content, blood, brain and kidney in 4-day neonatal rats.

Sturtz *et.al*. has very limited value for human risk assessment. The nominal 2,4-D treatment rates to the lactating rats were 50, 70 and 100 mg/kg bw/day, doses in excess of saturation of renal clearance of 2,4-D (van Ravenzwaay *et al*., 2003; Saghir *et al.*, 2006; 2009). The toxicity observed in the resulting non-linear pharmacokinetic range does not inform human risk assessment (EPA RED), because nonlinear doses are exceedingly unlikely to occur in humans. The Sturtz *et al.* studies have some significant methodological problems, and appear to rely on inappropriate statistics (combining male

and female pup weights but analyzing changes by individual pup and not litter mean)]. Thus Sturtz *et al.* is not useful in assessing any potential human risk from substantially lower occupational and general population exposures to 2,4-D, including infants.

In the development of the 2,4-D RED, EPA was well aware of the potential for 2,4-D to be transferred to nursing offspring through milk and previously addressed NRDC's concerns: "EPA is aware, as a result of animal feeding studies using exaggerated doses, that 2,4-D may be present in milk. It is not surprising that the study relied upon by NRDC suggests that 2,4-D is transmitted in breast milk given the massive doses of 2,4-D in that study of 50, 70 and 700 milligrams/kilogram of body weight/day (mg/kg/day). By comparison, EPA estimates that the maximum dietary exposure from food to human females ages 13-50 is 0.01018 mg/kg/day and the average exposure is 0.000642 mg/kg/day (USEPA Memorandum, 2002). These values range from 4,900 to 1 million times lower than the values in the cited rat study. Further, EPA's manner of doing risk assessment for infants is protective of any pesticide exposure to infants from human breast milk because the exposure values EPA assumes for pesticides in cow's milk greatly exceed the values that could be present in breast milk." (US Federal Register 2005 at 46706, 46735).

The EPA estimates of average human 2,4-D exposures described above are demonstrably reasonable. Recent human biomonitoring of farm families, including non-applicator spouses and children during a period of high 2,4-D use (spring time farm application period), established a geometric mean dose of 0.00008 mg/kg/day for the spouses and 0.00022 mg/kg/day for all children (Alexander *et al.*, 2007). These very low-level exposures have been confirmed in an EPA-conducted biomonitoring study of 135 pre-school children and their adult care-givers from randomly selected households in North Carolina and Ohio. Despite detection of 2,4-D in samples of carpet dust from most of the surveyed households, the highest level of exposure found in a single child was 0.00028 mg/kg/day, while the 50th percentile value in the children was ten-fold lower (Morgan *et al.*, 2008).

For both the occupational and indoor residential exposures characterized above (Alexander *et al*., 2007; Morgan *et al*., 2008), a low potential to adversely impact human risk has been supported in a recent analysis by Aylward and Hays (2008, 2010). Using animal and human pharmacokinetic information, these investigators calculated Biomonitoring Equivalent (BE) values for 2,4-D exposure, which were defined as urine or blood concentrations of 2,4-D expected to result from human exposure in relation to existing health-based exposure guidelines. For 2,4-D, urine and plasma BE values were derived for exposures to both chronic and acute reference doses (RfD). Both the occupational and general population urine concentrations reported in Alexander *et al.* (2007) and Morgan *et al.* (2008) are well below even the lowest BE calculated for the EPA chronic RfD, and even further below BE's estimated for acute exposures, *i.e.,* values appropriate for reference to occupational or short-term residential exposures. Urinary biomonitoring samples reflect aggregate exposure inclusive of both occupational and other incidental exposure sources, *e.g.,* house-dust, and surface contamination from outdoor to indoor track-in. Thus, these data also indicate that the presence of 2,4-D in household dust or exposure scenarios as noted in the Nishioka *et al.* (1996) study cited by NRDC or in Morgan *et al*. (2008) does not translate to absorbed doses of 2,4-D likely to present an adverse human health risk. In summary, the presence of a detection in dust does not equate to risk. In contrast, comparison of the biomonitoring findings with the BE calculated for 2,4-D indicated that there was a considerable margin of safety in the biomonitored populations, which included 2,4-D applicators and their families measured during the seasonal periods of 2,4,-D application.

NRDC also claimed that, "EPA failed to include any lactational exposure in its aggregate risk assessment." That statement is not correct. The language used in the EPA 2,4-D Health Effects Division risk assessment states in section 4.2.2 -- Acute Dietary Exposure and Risk, 'The acute dietary assessment was slightly refined as the following fairly conservative assumptions were made: … Note that ½ of the average LOD from PDP monitoring data was used as the milk exposure value because no milk samples contained detectable 2,4-D residues over several years of PDP sampling" (USEPA Memorandum 2004b). For the 2,4-D risk assessment, EPA assumed that 2,4-D would be present in

milk at 0.004 ppm for both acute and chronic exposure (despite it being non-detectable in PDP sampling). EPA's aggregate exposure assessment was protective for all children, including nursing infants.

NRDC further alleges "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," and cites the 2006 report of Sturtz, *et al*. However, the decreased pup body weights reported in litters of dams treated with 15 mg/kg/day of 2,4-D in the diet were not replicated in the range-finding study for the F1-extended one generation study conducted under Good Laboratory Practice (GLP) conditions (Saghir *et al.* 2008a) demonstrated that 2,4-D significantly decreased pup body weights only when dams were treated with 2,4-D equal to or greater than 800 ppm in the diet continuously from pre-breeding through completion of lactation on postnatal day 21 (equivalent to 52, 61, and 71-117 mg/kg/day during pre-breed, gestation and lactation periods respectively). In the definitive study, pup weights were also affected at 600 ppm (Marty *et al*., 2010), which was a dose above the KMD. Pup body weights were not affected in litters from dams at 400 ppm (25, 28 and 37-57 mg/kg/day, respectively) (Marty *et al*., 2010). Importantly, these investigators also demonstrated that the body weight decreases noted at ≥600 ppm occurred under conditions in which 2,4-D pharmacokinetic behavior in the lactating dams was distinctly non-linear, thus rendering these responses of limited relevance to human risk assessment (Saghir *et al.*, 2008a; Marty *et al*., 2010).

NRDC adds a minor discussion on potential effects on the brain of neonatal rats exposed lactationally to 2,4-D, citing recent 2006 and 2007 papers by the same research group in Argentina. These studies were conducted at high doses of 2,4-D (well above pharmacokinetic nonlinearity), and included use of an unrepresentative route and mode of administration (intraperitoneal) which would be expected to further amplify nonlinear pharmacokinetic performance (Garcia *et al.*, 2004; Ferri *et al*., 2007;Garcia *et al*., 2006).

The Industry Task Force II on 2,4-D Research Data has completed the F1-extended one-generation reproduction study that examined potential life-stage impacts of dietary 2,4-D administration to rats, including evaluation of developmental neurotoxicity and brain

development. There were no exposure-related effects on brain morphometrics or myelin deposition (assessed through special staining), or on developmental neurotoxicity parameters. (Marty *et al*., 2010).

Allegation: Underestimated dermal absorption factor

For dermal absorption risk assessment NRDC claims that EPA used a dermal absorption factor of 10 percent which is inappropriate considering the synergistic effects of other exposures.

Among the studies EPA considered to establish the dermal absorption risk factor was a Feldmann and Maibach (1974) human study that showed absorption of 5.8% ± 2.4 (3.4-8.2%) of 2,4-D applied to the forearm skin. This study was an extreme test using ^{14}C 2,4-D applied with an acetone vehicle. Acetone tends to denature the skin, a process in which proteins or nucleic acids of the skin lose their 3D-structure, thus allowing for increased potential dermal absorption. The skin was not protected and area not washed for 24 hours for maximum absorption.

Recently, Ross *et al.* (2005) reviewed and summarized numerous dermal absorption studies and determined that the human percutaneous absorption of 2,4-D has been well characterized. These investigators examined five published studies using human subjects, including Feldmann and Maibach (1974), and concluded these studies exhibited remarkable reproducibility across a span of three decades and multiple laboratories, formulations, and methods. They also noted that while it is considered rare to have even two human dermal studies available to assess dermal absorption of a specific chemical product, having five 2,4-D studies was exceptional. These human data provide valuable perspective for characterizing the variability (CV=60%) and central tendency (mean = 5.7% dermal absorption) across the published studies, and supported the conclusion that the central tendency value, which was in close agreement with the finding of Feldmann and Maibach (1974), can be used with confidence in human risk assessment. Ross *et al.* further observed that the human dermal absorption determinations predicting a low

percentage of 2,4-D dermal absorption were in excellent agreement with findings from worker biomonitoring studies in which measured internal doses were very low and ranged from 2.0-5.2 μg/kg body weight. In addition to the comprehensive analysis of Ross and co-workers, EPA has previously acknowledged that, although the literature denotes a 2,4-D dermal absorption of 5.8%, a 10 percent dermal absorption factor was selected as a protective value given study variation (USEPA 2005).

Allegation: Dermal absorption enhanced by other factors

NRDC alleges that the dermal absorption of 2,4-D may be elevated in the presence of several interacting factors such as use of sunscreen, alcohol consumption and the insect repellent DEET. EPA has specifically responded to this allegation in the past as follows:

> *"With regard to the dermal absorption value used and the use of insect repellents and/or sunscreens, in reality, those same farmworkers describe[d]… as not having the opportunity to change their work clothes and/or shower, most likely would not be using these potential enhancer products either. [For the Moody et al., 1992, study specifically examining DEET]… the conclusion that the data demonstrate a difference between exposure with [14±4.5%] and without [10±11.5%] DEET is not supported given the magnitude of the standard deviation. What can be gleaned from the study is that significant exposure can occur from hand contact, and taking measures to limit dermal exposure; e.g., washing after exposure and or the use of chemical-protective gloves, is recommended*."

Concerns for potential interacting substances to impact dermal absorption has been further mitigated by personal protective equipment (PPE) changes implemented by EPA in the 2005 re-re-registration document (RED) of 2,4,-D (USEPA 2005, p.114). The RED describes the PPE requirements for liquids, wettable powder, formulations in water-soluble packages and water-dispersible granules as:

"All mixers, loaders, applicators, flaggers, and other handlers must wear:

- long-sleeved shirt and long pants,

- shoes and socks, plus

- chemical resistant gloves, when applying postharvest dips or sprays to citrus, applying with any handheld nozzle or equipment, mixing or loading, cleaning up spills or equipment, or otherwise exposed to the concentrate.

- chemical resistant apron when applying postharvest dips or sprays to citrus, mixing or loading, cleaning up spills or equipment, or otherwise exposed to the concentrate."

The requirement to use chemical resistant gloves in demonstrated conditions of intensive exposure potential has in fact been demonstrated to significantly mitigate 2,4-D exposures. The EPA RED decision regarding glove use in high-potential exposure conditions is consistent with a robust peer-reviewed literature which has repeatedly demonstrated that use of protective rubber gloves alone dramatically reduces the total absorbed dose 2,4-D (Alexander *et al*., 2007; Arbuckle *et al.*, 2002; Hines *et al.*, 2001; Harris *et al.*, 2002). The focus on hand protection is also consistent with a report that concluded contact with hands accounted for 80-90% of potential cumulative worker exposure to 2,4-D (Grover *et al.*,1986). Thus, in addition to EPA's previous comments addressing why the proposed chemical interactions are unlikely to impact dermal absorption, it is also clear the potential impacts of such postulated interactions would be significantly reduced by the EPA RED- mandated call for use of chemical-resistant gloves in high-intensity exposure conditions.

Allegation: Rubber glove permeability is enhanced by DEET and sunlight

NRDC infers that glove use will not afford adequate dermal protection by citing a study (Moody and Nadeau, 1992) as concluding that simultaneous exposure of rubber gloves to "DEET and sunlight" rendered them "highly permeable" to 2,4-D penetration. NRDC then postulated that if enhanced penetration occurred it would be associated with increased dermal absorption due to glove occlusion of the skin.

Moody and Nadeau (1992) examined total ^{14}C-ring labeled 2,4-D permeability across a 1.5 cm^2 cut-out of natural rubber latex gloves incubated in laboratory apparatus at 37º C for 48 continuous hours. This study was primarily intended as an hypothesis-

generating experiment and does not provide any definitive conclusions for enhanced risk of 2,4-D dermal penetration associated with glove use for the following reasons:

1) although the NRDC petition infers that DEET alone enhanced 2,4-D permeability, the authors concluded DEET did not significantly impact 2,4-D penetration through the rubber (2.4 ± 1.8% with DEET vs 3.2 ± 3.46 without DEET);

2) in what can only be regarded as preliminary evidence (n = 2), the permeability of 2,4-D was increased to 6.2 ± 0.73% when the rubber was simultaneously exposed to a UV lamp emitting UVA (not "sunlight"; non-UVA control 0.3 ± 0.14% 2,4-D permeation);

3) neither DEET alone (no effect on permeation) or UVA alone rendered the rubber "highly permeable" to 2,4-D despite the relatively severe experimental conditions;

4) only permeation of total radioactivity was measured and thus the authors themselves could not exclude that the postulated enhanced permeation with UVA-co-treatment was represented by 2,4-D photolysis product(s); and

5) the authors stated the field relevance of the observations remained to be established.

As noted above, however, several well-conducted studies have demonstrated that use of rubber gloves in fact significantly reduced 2,4-D dermal absorption in actual field conditions. These exposure reductions occurred even when no special precautions were taken to assure use of undamaged gloves (Alexander *et al*., 2007; Arbuckle *et al*., 2002; Hines *et al*., 2001; Harris *et al*., 2002).

Allegation: Dermal penetration implications of occlusion and/or soaking of 2,4-D into clothing not adequately assessed

As summarized above, repeated field studies have confirmed that protection ("occlusion") of skin by use of rubber gloves dramatically reduces 2,4-D dermal absorption.

In a comprehensive study, Lavy, *et al.* (1987) and co-workers examined the overall absorption of 2,4-D in four types of intensive exposure 2,4-D forestry applications: backpack, injection bar, hypohatchet, and hack-and-squirt. For each of the four types of exposure scenarios, 2,4-D absorption was evaluated in four crews of 20 workers each under two exposure conditions: 1) workers wore usual clothing but no additional PPE and followed normal work habits; and 2) workers were issued new leather gloves and leather boots for spraying and were also required to use all feasible precautions to reduce exposure (*e.g.*, use neoprene gloves for mixing/filling; wash hands before rest periods; bathe and change clothes as soon as possible after work). For all types of applications except backpacks, use of new gloves, boots and other precautions reduced overall 2,4-D absorption. Use of the additional exposure precautions did not, however, impact 2,4-D exposures in backpack applicators. Although 2,4-D exposure was highest in these applicators and was attributed to the observation that the clothing of both groups often was saturated with spray, dew or perspiration, it is important to note that even despite the extreme exposure conditions associated with forestry backpack application the average applicator exposures averaged 0.0876 – 0.0980 mg/kg. These exposures were not considered health threatening by the authors in that they estimated a margin of exposure of 272 relative to the referent animal toxicity NOEL of 24 mg/kg/day. Thus, a low dermal absorption potential for 2,4-D was demonstrated even under conditions of intensive exposure such as that represented by saturation of clothing, Agricultural and lawn care applicator scenarios also have been shown to result in dermally absorbed doses that are significantly less than forestry backpack applicators. For example, the geometric mean exposure to 2,4-D farm applicators has been reported as 0.00246 mg/kg/day (2.46 µg/kg/day; Alexander *et*

al., 2007), while professional lawn care applicators exposure did not exceed 0.006 mg/kg/day (6 µg/kg/day; Yeary, 1986) and homeowners applying liquid 2,4-D formulation with no gloves or other PPE precautions (and evidence of direct spills of concentrate on bare skin during applications) experienced a maximum 2,4-D dose of 0.0071 mg/kg (7.1 µg/kg; Harris *et al*., 1992). Importantly, homeowners using recommended PPE such as rubber gloves and/or using granulated formation experienced mostly non-detectable exposure (Harris *et al*., 1992).

Allegation: When tracked indoors, 2,4-D persists in carpets for up to one year after a single turf application.

NRDC states that 2,4-D tracked into homes persists in carpets for up to one year at concentrations of 0.5 µg/gm (Nishioka *et al.*, 1996). Nishioka *et al.* (1996) calculated that value for carpet dust from short-term carpet dust samples collected in a tracking study. The value of 0.5 µg/gm is higher than the average 2,4-D concentration of 0.156 µg/gm found in carpet dust samples collected in Ohio homes of 2,4-D applicators (Morgan *et al.,* 2008) and substantially higher than the average level of 0.0475 µg/gm found in similar applicator's homes in North Carolina. Morgan *et al.* (2008) reported exposure levels of spouses and children of the applicators and found the maximum exposure for a child to be 0.00028 mg/kg/day while the 50th percentile exposure in children was ten fold lower than that. These exposures are substantially below the oral reference dose for 2,4-D.

CONCLUSION

Data quality considerations and balanced reviews are critical. 2,4-D is a longstanding product and there are numerous published papers in the literature that can be 'cherry picked' to allege 2,4-D effects. Emphasis must be placed on high quality, studies that have been conducted using validated protocols, standardized guidelines, and Good Laboratory Practice Standards including the most recent F1-Extended One Generation Reproduction study, (Marty *et al*., 2010 MRID 47972101). As described previously, this study included a comprehensive analysis of endocrine and DNT endpoints (as well as systemic toxicity and developmental immunotoxicity) and did not identify exposure-related adverse effects below the KMD, except previously known renal toxicity. Importantly, the F1-extended one generation study provided no support for a lower NOAEL than previously established. The study supports removal of the currently applied 10x database uncertainty factor, and does not support the need for an FQPA safety factor. Peer-reviewed, replicated studies from the scientific literature can also be of value. However, open literature cited in the NRDC petition are of questionable utility due to limitations in experimental design, limited exposure documentation, lack of dose response, excessive doses (above renal saturation threshold), absence of positive controls and misleading conclusions, especially as re-interpreted by NRDC. The NRDC petition should be denied.

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