

Regulatory progress, toxicology, and public concerns with 2,4-D: Where do we stand after two decades?

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Abstract

2,4-D is member of the phenoxy family of herbicides and has major uses in agriculture, forestry, turf, non-crop and aquatic weeds. Since its introduction in 1946, the toxicology of 2,4-D has been studied extensively and repeatedly. Beginning in 1980, regulatory agencies in North America and Europe initiated re-registration/re-evaluation activities for 2,4-D, which resulted in the formation of the Industry Task Force II on 2,4-D Research Data, and has resulted in the submission of 60 toxicology studies conducted to GLP standards using 2,4-D acid and its dimethylamine salt and 2-ethylhexyl ester forms. The various forms of 2,4-D were toxicologically equivalent. 2,4-D in all three forms has low-to-moderate acute oral toxicity (rat LD₅₀ 699–896 mg/kg) and is not well absorbed through skin. In rat and mouse subchronic and chronic studies, overall dietary no-observed-adverse-effect-levels (NOAEL) were 15 and 5 mg/kg/day, respectively. 2,4-D was not carcinogenic in either rodent species, consistent with a lack of genotoxicity in *in vitro* and *in vivo* test systems. Mild kidney toxicity was the primary toxic effect in these studies. 2,4-D was not a developmental toxicant in rat (overall NOAEL 25 mg/kg/day) and rabbit (overall NOAEL 75 mg/kg/day) studies, had a low potential for multi-generation reproductive toxicity and neurotoxicity (NOAELs 5 mg/kg/day, respectively). When compared to estimated human exposure levels, the overall toxicology NOAEL of 5 mg/kg/day represents a margin of exposure (MOE) of 1700 for commercial applicators and 50,000 for home and garden users. Thus, coupled with the extensive toxicology data, 2,4-D meets safety standards for all countries where it is registered. Additional 2,4-D information is available on the Industry Task Force II on 2,4-D Research Data website www.24d.org.

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1. Introduction

2,4-Dichlorophenoxyacetic acid (2,4-D) is member of the phenoxy family of herbicides and has major uses in agriculture crops, forestry, turf, non-crop and aquatic weeds. Since its introduction in 1946, the toxicology of 2,4-D has been studied extensively and repeatedly. Beginning in 1980, regulatory agencies in North America and Europe initiated re-registration/re-evaluation activities for 2,4-D which resulted in the formation of the Industry Task Force II on 2,4-D Research Data, and has also resulted in submission of 60 toxicology studies conducted

to good laboratory practice (GLP) standards using 2,4-D acid and its dimethylamine salt and 2-ethylhexyl ester forms.

The toxicology and human health effects of 2,4-D have been extensively reviewed (Munro et al., 1992; Bus and Leber, 2001; Gingell et al., 2001; Kennepohl and Munro, 2001; Garabrant and Philbert, 2002). Although there are thousands of studies describing the potential toxicity and health effects of 2,4-D, the purpose of this review is to overview the findings of animal and human health studies primarily conducted or sponsored by the Industry Task Force II on 2,4-D Research Data (2,4-D Task Force) with the three forms of 2,4-D (acid, dimethylamine salts, and 2-ethylhexyl ester). These studies serve as key data elements used by regulatory agencies to assess the potential human health risks associated with the use of 2,4-D.

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2. Acute toxicology

The acute oral toxicity of 2,4-D in rats is represented by LD₅₀ values of 699, 949 and 896 mg/kg, respectively, for the acid, salt and ester forms (Munro et al., 1992; Bus and Leber, 2001; Gingell et al., 2001). At these high dose levels clinical signs include ataxia and myotonia. In rabbits, LD₅₀ values of all three forms of 2,4-D following dermal application were all greater than 2000 mg/kg (Munro et al., 1992), and are consistent with a reported low skin absorption of less than six percent (Kennepohl and Munro, 2001, Maibach and Feldmann, 1974; EPA, 2004). Lethality also is not seen in rats following acute inhalation exposures, with LC₅₀ values greater than 1.8, 3.5 and 5.4 mg/L for the respective forms of 2,4-D (Munro et al., 1992).

All forms of 2,4-D are slight to minimal skin irritants in standard rabbit skin irritation tests. In addition, 2,4-D is not reported as a skin sensitizer in guinea pig sensitization studies (Kennepohl and Munro, 2001; EPA, 2004).

Both the acid and dimethylamine salts are severe eye irritants while the ester form is a minimal eye irritant (Munro et al., 1992; Gingell et al., 2001; Kennepohl and Munro, 2001; EPA, 2004).

3. Subchronic and chronic toxicity including carcinogenicity

The subchronic and chronic toxicity of 2,4-D has been characterized in rats, mice and dogs. All studies included evaluation of an extensive series of test parameters during and at termination of the studies. Parameters studied included: clinical observations, body weights, food consumption, ophthalmoscopic examinations, hematology and clinical chemistries, gross necropsies, organ weights, and complete organ histopathology evaluations.

In 90-day subchronic studies, rats were administered either 2,4-D acid and its salt and ester forms daily in the diet at 0, 1, 15, 100 or 300 mg/kg/day, salt and ester forms given as acid equivalents to 2,4-D (Charles et al., 1996a). Minimal histological alterations in kidney, liver, testes, and adrenals were mostly observed at the top dose level, a dose clearly that exceeded a maximum tolerated dose (MTD) as represented by body weight gain depressions of 37–88 percent of control values. Retinal degeneration was noted only in female rats treated with 300 mg/kg/day. Based on minimal histological alterations and minimal changes in hematology and clinical chemistry responses (red blood cell mass; thyroxin T_3 and T_4 values, platelet counts), the overall no observed adverse effect level (NOAEL) dose for these studies was 15 mg/kg/day. Importantly, comparable toxicity was observed for all three forms of 2,4-D. The slight effects on thyroid hormone levels, which were limited to high - doses of 2,4-D, suggest 2,4-D has only minimal potential to alter thyroid endocrine function.

In chronic toxicity/carcinogenicity studies, rodents were administered the 2,4-D acid form only daily in the diet, female and male rats at 0, 5, 75 and 150 mg/kg/day, and

female and male mice at respective dose levels of 0, 5, 150 and 300 mg/kg/day and 0, 5, 62.5 and 125 mg/kg/day (Charles et al., 1996b). Paralleling observations in the subchronic toxicity studies, 2,4-D exhibited a low potential for chronic toxicity, with minimal effects noted in rats in kidney, liver, thyroid, and eyes that were limited to the high-dose levels. Both the rat and mice studies found no evidence of carcinogenicity, even though the top dose groups of both studies achieved MTD dose definitions. The overall NOAEL value for the combined rat/mouse studies was 5 mg/kg/day. Mild effects in kidneys were common to both species.

The findings of the carcinogenicity studies reported above have been evaluated by several regulatory and international agencies. The United States Environmental Protection Agency (EPA) concluded “2,4-D acid was not carcinogenic in male or female Fischer 344 rats... (and) was not carcinogenic in male and female B6C3F1 mice.” (EPA, 1996). Both the World Health Organization (WHO) and the European Union Commission Health and Consumer Protection Directorate (EU) stated “There was no evidence of carcinogenicity ...in mice...(and) in rats.” (WHO, 1996; EU, 2001).

In genotoxicity assays, all of the various 2,4-D forms tested negative in genotoxicity assays including an in vivo mouse micronucleus assay (Charles et al., 1999a), Salmonella (Ames) reverse mutation assays both with and without metabolic activation (Charles et al., 1999b), and a rat hepatocyte unscheduled DNA synthesis assay (Charles et al., 1999b). In addition, 2,4-D did not produce chromosomal aberrations in primary cultures of rat lymphocytes, or forward mutations at the hypoxanthine guanine phosphoribosyl transferase (HGPRT) locus of Chinese hamster ovary cells (Gollapudi et al., 1999). The lack of carcinogenicity of 2,4-D in chronic animal toxicity studies is consistent with the overall lack of genotoxicity for 2,4-D and its salt and ester forms.

In a number of epidemiologic studies, conducted during its lengthy and extensive use in agriculture, 2,4-D has been evaluated for potential carcinogenicity in humans. Despite early associations of 2,4-D use with non-Hodgkin's lymphoma, a comprehensive review and evaluation of the epidemiologic literature concluded: “Epidemiologic studies provide scant evidence that exposure to 2,4-D is associated with soft tissue sarcoma, non-Hodgkin's lymphoma, Hodgkins disease, or any other cancer.” (Garabrant and Philbert, 2002). This conclusion is consistent with a recent study conducted by the United States National Cancer Institute (NCI), in which a large case control study of farmworkers in the American Midwest potentially exposed to 2,4-D found no evidence of an association between non-Hodgkin's lymphoma and “ever having used 2,4-D” (DeRoos et al., 2003).

Hayes and coworkers at the US NCI described a case control study claiming a link of residential use of 2,4-D to malignant lymphomas outcomes in pet dogs with access to treated areas (Hayes et al., 1991). However, a re-evaluation

of the raw data from this study did not confirm either a dose–response relationship or actual association between 2,4-D use and occurrence of canine malignant lymphoma (Kaneene and Miller, 1999).

Knowledge of the dose and species-dependent pharmacokinetic behavior of 2,4-D significantly enhances the understanding of the relevance of toxicity findings of 2,4-D in rodents, and particularly in dogs, to predicting potential human health risks. Once absorbed, 2,4-D is rapidly and completely excreted in urine by both rats and humans, but not dogs (Van Ravenzwaay et al., 2003; Timchalk, 2004). In rodents and human, renal excretion of 2,4-D is facilitated by a saturable organic anion active transporter located in the renal tubules (Timchalk, 2004). The transporter does not effectively function in dogs. Studies in rats indicate the renal clearance of 2,4-D is clearly saturated at oral dose levels of 50 mg/kg, resulting in non-linear increases in 2,4-D blood concentrations at this dose and above (Gorzinski et al., 1987; Van Ravenzwaay et al., 2003). Given this non-linear behavior, saturation of 2,4-D renal clearance at 50 mg/kg suggests that animal toxicity findings observed at this dose level and higher overestimate potential human risks. In the case of dogs, both subchronic and chronic studies indicate this species, with an overall NOAEL of 1 mg/kg/day (Charles et al., 1996c), is more sensitive to 2,4-D-induced toxicity than rodents, with an overall NOAEL of 5 mg/kg/day (Charles et al., 1996b). Since the dog is lacking an effective renal organic anion clearance mechanism, this differential species response has been attributed to an inability of the dog to effectively clear 2,4-D from the body, resulting in significantly higher 2,4-D blood concentrations in dog relative to rats and humans at an equivalent oral dose of 5 mg/kg (Van Ravenzwaay et al., 2003; Timchalk, 2004). Recently, the EPA has concluded, that the rat represents a better predictor of potential toxicity in man than the dog (EPA, 2004).

4. Teratogenicity, reproductive toxicity and neurotoxicity

The potential for 2,4-D to produce birth defects and alter reproductive and neurological function also has been assessed in a series of GLP-quality studies. The teratogenicity of 2,4-D and its various salt and ester forms has been evaluated in both rats and rabbits (Charles et al., 2001). Mild fetal developmental toxicity was observed only at doses which also produced evidence of maternal toxicity, indicating developing rats and rabbits fetus are not uniquely sensitive to 2,4-D toxicity. The overall NOAEL for developmental toxicity in these studies was 25 mg/kg/day.

The potential 2,4-D reproductive toxicity has been assessed in a two-generation reproduction study in rats (oral dietary doses of 0, 5, 20 and 80 mg/kg/day; summarized in Munro et al., 1992; Bus and Leber, 2001; Gingell et al., 2001). The top dose resulted in excessive toxicity in the offspring and was not further evaluated. Minimal decreases in pup body weights in an absence of

effects on fertility were reported at the mid-dose level, resulting in an overall reproductive toxicity NOAEL of 5 mg/kg.

The neurotoxicity potential of 2,4-D has been evaluated in both a single-dose acute and a 1-year chronic dietary study in rats (Mattsson et al., 1997). These studies included assessments of a functional observational battery, motor activity, and comprehensive neurohistopathology of perfused tissues. In the acute neurotoxicity study, 2,4-D acid was administered at nominal dose levels 0, 15, 75 and 250 mg/kg (0, 13, 67 and 227 actual). The top dose of 227 mg/kg caused slight transient alterations in gait and coordination and decreased motor activity 1-day after treatment that was fully reversible by day 8 post-treatment. The overall acute NOAEL was established as 67 mg/kg based on a mild locomotor response noted in a single animal at the mid-dose of 67 mg/kg. In the chronic dietary assessment the study NOAEL was 75 mg/kg/day based on retinal degeneration in high -dose, 150 mg/kg/day female rats.

5. Margins of exposure (MOE) between animal toxicity findings and human exposures

Characterization of the potential human risks to pesticide exposures can be estimated by calculation of MOEs, the ratio of NOAEL values obtained from animal toxicity studies to estimated human exposures. As shown in the information above, the overall lowest NOAEL from animal toxicity studies is 5 mg/kg/day based on the findings from chronic rat and mouse studies. Since 2,4-D is completely and rapidly excreted in urine in humans, collection of total 24-h urine samples provides reasonable estimates of immediate 2,4-D exposures. For professional workers employed as commercial yard sprayers, total 2,4-D exposure has been estimated as 0.003 mg/kg/day, resulting in a calculated MOE of 1700 (Yeary, 1986). For non-professional home and garden 2,4-D users, exposure is estimated at 0.0001 mg/kg, resulting in an MOE of 50,000 (Solomon et al., 1993). In both cases the large MOE between a dose causing no toxic effects in animals and actual estimates of human exposures under real-world use conditions suggest a high margin of safety for approved uses of 2,4-D.

6. Summary

Studies conducted or sponsored by the Industry Task Force II on 2,4-D Research Data provide a package of GLP-quality toxicity studies that characterize the range of toxicity potential of 2,4-D as required by the United States and European regulatory authorities. Details of these studies and others can be obtained at the 2,4-D Task Force website, www.24d.org. Overall, these studies indicate that 2,4-D has only low-to-moderate toxicity. Chronic and other toxicity responses are generally limited to high doses, well above those known to result in non-linear

pharmacokinetic behavior. 2,4-D is not an animal carcinogen or genotoxicant, does not cause birth defects, and has low potential for reproductive toxicity and neurotoxicity. The various acid, salt and ester forms of 2,4-D show toxicologic equivalence. Thus, 2,4-D meets safety standards for all countries in which it is registered.

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