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A Comprehensive, Integrated Review and Evaluation of the Scientific Evidence Relating to the Safety of the Herbicide 2,4-D[1]

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Preface

The herbicide 2,4-D has been commercially available throughout the world for nearly fifty years, having been first registered for sale in the U.S. in 1948. During that time, it has come to be one of the most relied upon and best studied of all agricultural chemicals. A 1978 scientific review stated that more than 40,000 scientific articles and technical reports addressing 2,4-D had been published at that time.

Questions were first raised about the safety of 2,4-D and the other phenoxy acid herbicides with the publishing of a series of case-control studies by Swedish investigator Lennart Hardell during the late 1970s. In these studies, Dr. Hardell hypothesized that exposure to this class of herbicide, as well as dioxins that were known to contaminate 2,4,5-T, was associated with the occurrence of three rare forms of cancer: Hodgkin's disease (HD), soft tissue sarcoma (STS), and non-Hodgkin's lymphoma (NHL). Attention to these herbicides was also sharpened in the public eye during the late 1970s because phenoxy acid herbicides were primary constituents of Agent Orange, the herbicide mixture used extensively as a defoliant during the Vietnam war.

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[1]This report has been peer reviewed by a panel of scientists chosen for their expertise in toxicology and epidemiology. The panel members were Sir Richard Doll, FRS, FRCP, Oxford University; John Doull, M.D., Ph.D., Kansas State University; Saxon Graham, Ph.D., SUNY at Buffalo; Raymond Greenberg, M.D., Ph.D., Emory University; and Gary Williams, M.D., American Health Foundation.

In 1979, the dioxin-contaminated phenoxy herbicide 2,4,5-T was suspended by the U.S. Environmental Protection Agency (EPA) (Josephson, 1980; Easley *et al.*, 1983). Also at that time, the EPA considered taking similar action with respect to 2,4-D. While calling for more data, the EPA stated that:

> "...EPA believes that available information on potential adverse health effects of 2,4-D does not warrant a regulatory action to remove its products from the market. The agency also does not see an imminent hazard or unreasonable health effects when 2,4-D products are used according to label instructions and precautions." (Josephson, 1980)

Controversy with respect to 2,4-D surfaced again in 1986 when investigators from the National Cancer Institute (NCI) published information from a case-control study related to farming. While there were no data to support an association between farming practices including the use of herbicides and either HD or STS, these investigators reported an association between suspected use of herbicides in Kansas farming for more than 21 days per year and NHL.

Following the publication of these data, independent reviews of the study were commissioned by the EPA, the Ontario Ministry of Environment, Agriculture Canada, and the Council for Agricultural Science and Technology. Each of these independent reviews of the studies and related data came to essentially the same conclusion: that the cancer risk hypothesis was not borne out by data in the NCI study and that continued use of 2,4-D in agriculture did not pose an unreasonable risk to public health.

In 1990, the same investigators from NCI published data that suggested that among Nebraska farmers there was an increasing risk of NHL with increasing usage of 2,4-D as measured by reported days of use per year. Following the release of this information, a blue-ribbon panel review of the scientific evidence regarding 2,4-D health risks was convened by the Harvard School of Public Health. While the main focus of the panel review was data generated by the NCI investigators, information on more than a dozen other epidemiological studies conducted around the world relative to 2,4-D and cancer was also considered. The report of this panel, published in 1991, concluded that any link between 2,4-D and cancer was far from established.

In 1991, investigators from NCI published yet another study allegedly implicating 2,4-D as a cancer

hazard: a case-control study of malignant lymphoma among dogs whose owners reported the use of herbicides. A panel review of that study commissioned by the Industry Task Force II on 2,4-D Research Data concluded that due to limitations in the study design, the NCI dog study did not show an association between owners' 2,4-D use and malignant lymphoma in dogs.

In the spring of 1992, NCI investigators published a third case-control study of farmers that reported small risks of NHL associated with the use of 2,4-D, although these risks did not appear to increase with latency or failure to use protective equipment. The publication of these studies collectively has led the EPA to announce that it will convene a scientific review panel to again review the weight of scientific evidence regarding the herbicide 2,4-D, which now includes numerous epidemiological studies in addition to those conducted by NCI researchers as well as hundreds of toxicological and other relevant studies in laboratory animals. This panel is expected to be convened in the fall of 1992.

The report that follows is the most comprehensive gathering and evaluation of the scientific evidence relating to the safety of 2,4-D completed to date. Several reviews of this scientific literature have been completed over the years; many of those previous reviews are cited in this report. However, the current report is unique in that it goes beyond the scope of a traditional review of the literature and encompasses an integrated approach to evaluating the weight of scientific evidence regarding the safety of this compound.

The authors undertook an in-depth review and analysis of all the relevant literature bearing on the safety of 2,4-D. The report is organized into several sections, each addressing a body of scientific information essential to addressing the above question. A rationale for interpreting the data within the context of the total weight of scientific evidence is included. Key studies are discussed in detail and other relevant literature contributing to the report is cited.

INTRODUCTION

Purpose of review

Since 1980, the manufacturers of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) have conducted, sponsored, or otherwise reviewed hundreds of scientific studies bearing on the safety of the

herbicide. These activities have been conducted in part as a response to U.S. Environmental Protection Agency (EPA) requirements for re-registration of the herbicide for use in agriculture, forestry and home lawn care and, in part, out of responsible concern for the safety of those exposed to their products.

During the spring of 1992, the EPA announced its intention to publicly review the scientific information that has been developed over the past decade relative to 2,4-D. This public review will be part of the process being followed by the EPA in deciding whether to place 2,4-D into formal Special Review status, which would be necessary if questions remained as to its safety.

The review reported here was commissioned by the manufacturers of 2,4-D in an effort to provide an up-to-date independent assessment of the weight of scientific evidence relative to the safety of the compound, as well as provide a vehicle through which the considerable scientific literature pertaining to 2,4-D can be consolidated for consideration by the EPA public review panel.

Framework for evaluating the safety of 2,4-D

In evaluating the potential health risk to humans from the herbicide 2,4-D, it is critical that toxicology, epidemiology, exposure, and biology information be considered integrally in the prediction of the nature of the possible effects as well as in the characterization of the dose-response relationships (Doull, 1989). In addition, it is generally agreed that the evaluation of human health risk should be based on the use of scientific judgement in the assessment of the weight of scientific evidence, rather than on quantitative analyses of data from selected studies (Burek *et al*., 1988; Clayson, 1987; Dybing, 1986; Johannsen, 1990; Koestner, 1986; Squire, 1981; Swenberg, 1986). Both of the above principles were incorporated into this review.

Overall, the value of an integrated weight-of-evidence evaluation resides in the ability to take advantage of the strengths of the respective scientific disciplines, while not being severely limited by the inherent weaknesses of specific studies within disciplines. While a limitation of epidemiology may be its inability to discern mechanisms of toxicity, the ability to discern mechanisms is a strength of toxicology. While toxicology requires prediction of effects in humans, epidemiology provides empirical evidence directly in humans. In addition, considering all relevant data provides a solid basis

from which to construct realistic assumption scenarios for exposure modeling, dose-response considerations, and assessing cause-effect relationships.

In this review of the scientific information on 2,4-D, safety was evaluated first with respect to the basic tenets of toxicology and epidemiology. Secondly, studies were evaluated in the context of integrating data according to scientific principles necessary to determine cause and effect relationships. The concept of causation is central to the effort of protecting public health, in that knowing the cause aids in identifying measures to prevent adverse health effects.

From the point of view of toxicology, the critical review of studies included consideration of the nature of the lesion, the dose-response relationship, sex-, species-, and site-specificities of the effect, historical control incidences, pharmacokinetics, metabolism, genotoxicity, and other relevant mechanistic data with particular emphasis on the evaluation of the relevance of experimental findings to humans.

A key factor in the assessment of relevance of effects in animals to humans is the establishment of a plausible mechanism of action by which humans could be affected at exposure levels of concern. Moreover, the consistency of the response among different studies and experimental conditions as well as a critical evaluation of the quality and shortcomings of each study were considered in this weight-of-evidence analysis.

From the epidemiological perspective, causal relationships or even direct associations are often difficult to establish because many potential biases can obscure true relationships between independent and dependent variables. In this review, every epidemiological study considered was critically evaluated from the perspective of identifying its strong and weak points. This critical review therefore provided the context within which each epidemiological study could be interpreted.

Among the points considered in the critical review of the epidemiology studies were:

- avoidance of bias in selecting members of the study group,
- verification of the exposure and the outcome,
- use of an appropriate comparison group,
- identification and control of confounding factors,

- magnitude of the observed association,
- statistical power to identify a health risk,
- whether the exposure and the outcome occurred in the proper temporal relationship,
- whether increases in exposure were associated with increases in the outcome (*i.e.*, evidence of dose-response),
- specificity of the association between exposure and effect,
- consistency of the findings within a given study,
- consistency of results between epidemiological studies, or replication of the findings,
- biological plausibility of the association, and
- consistency of the results between disciplines.

Following critical review of both the experimental toxicology data and the observational data from humans, the data on 2,4-D were integrated in order to address the question of 2,4-D health risk. The framework for integrating these data included: evaluation of pathways for exposure, metabolism of the compound, mechanisms through which adverse effects might be manifested, and consistency of findings across the scientific disciplines with special emphasis on concordance between epidemiological and toxicological findings.

HISTORY OF USE OF 2,4-D AND HUMAN EXPOSURE TO 2,4-D

Chemistry and uses of 2,4-D

The basic form of 2,4-D is 2,4-dichlorophenoxyacetic acid (CAS No. 94-75-7), but it is often formulated as an inorganic salt, amine or ester (WHO, 1984). The two primary approaches to constructing 2,4-D are condensation of 2,4-dichlorophenol with monochloroacetic acid or chlorination of phenoxyacetic acid (WHO, 1984). 2,4-D alkali metal salts are prepared by reaction of 2,4-D with a metal base, 2,4-D amine salts by reaction of 2,4-D with amine, and 2,4-D esters by acid-catalyzed esterification or direct synthesis of a monochloroacetic acid ester with dichlorophenol (WHO, 1984).

Plants absorb 2,4-D through their roots and leaves within four to six hours after application. Following absorption, 2,4-D progresses upward through the plant in the phloem (EPA, 1989). Within the plant, 2,4-D mimics the effect of the auxins, or plant growth-regulating hormones and stimulates growth,

rejuvenates old cells, and overstimulates young cells, which leads to an abnormal growth pattern and death in some plants (Mullison, 1987). Plant metabolism also is affected by 2,4-D through modification of enzyme activity, respiration, nucleic acid synthesis, protein synthesis, and cell division (EPA, 1989), and through congestion of the phloem, thus interfering with food transport (Mullison, 1987). 2,4-D is selectively toxic to broafleaf plants due to their larger leaf area, which leads to absorption sufficient to alter plant growth (Seiler, 1978).

2,4-D was first registered for use in the U.S. in 1948 (EPA, 1989). By 1983, approximately 1500 products containing 2,4-D were registered with the U.S. Environmental Protection Agency (Easley *et al.*, 1983). The annual use of the 2,4-D active ingredient in the U.S. was estimated in 1990 to be 52 to 67 million pounds (Archibald and Winter, 1990).

The primary use of 2,4-D is as a herbicide, due to its effectiveness at selectively eliminating broadleaf plants. 2,4-D is a mainstay in agriculture, forestry, and lawn care. It is estimated that over 75% of the total usage of 2,4-D in the U.S. is for weed control in agriculture, especially in wheat and corn fields (EPA, 1989). Additional uses of 2,4-D are in forestry, along rights-of-way, on rangelands, in parks, on golf courses, in aquatic situations, and, to a much lesser extent, for home lawn care and gardening (Ibrahim *et al.*, 1991). Some of the flora against which 2,4-D has been reported to have been used are: Eurasian water milfoil, water hyacinth (WHO, 1975); dandelion, plantain, chickweed, henbit, white clove, heal-all, sheep (red) sorrel, curly dock, chicory, yellow rocket, speedwell, ground ivy, spurge, oxalis, knotweed, purlsane, thistle, wild violet, wild onion, wild garlic, lespedeza, yellow nutsedge, crabgrass (Lefton *et al.*, 1991); and sumac, willow, sagebrush, and ragweed (Mullison, 1987).

A relatively minor category of use for 2,4-D is as a growth regulator. Examples of this type of use include prevention of premature dropping of fruit, favorable selection of the growth of medium-sized potatoes, enhancement of the color of potatoes (WHO, 1989), increased size of citrus fruits, and increased vitality of citrus fruits after harvest to retard fungal growth (WHO, 1975).

The companies of the Industry Task Force are planning to re-register 2,4-D for small grains use (including wheat), corn and sorghum use, industrial use, forestry use, pasture and rangeland use, turf use, and aquatic use. The crop uses not being supported for re-registration by the members of the Industry Task Force are apples, grapes, tree nuts, small fruits, potatoes, pears, stone fruits, citrus, and

berries. Some of these uses might be re-registered by other parties. Use on soybean fields prior to planting is a new use for 2,4-D that will be registered due to the urging of the American Soybean Association, the U.S. Department of Agriculture Soil Conservation Service, and other groups (Page, 1992).

Fifty-six million pounds of 2,4-D were used in the Vietnam war to defoliate mangrove forests and bean, peanut, ramie, root and tuber crops; this represented 53% of all herbicides used in the conflict. The best known 2,4-D-containing formulation from Vietnam was Agent Orange, which consisted of 2,4-D and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) in equal proportions. Agent Orange made up 10.7 million of the 17.7 million gallons of herbicides used in Vietnam (Wolfe, 1983).

Impurities and adjuvants of 2,4-D

Questions have been raised about the toxicity associated with non-active ingredients, such as solvents and emulsifiers, which are added to facilitate application of the active ingredient, 2,4-D. While some toxicology studies have specifically addressed the potential effects of possible contaminants such as chlorinated dioxins and furans, diesel oil, and other impurities, data gaps exist.

On the other hand, epidemiology studies that have been conducted on applicators, farmers and others who have sustained exposures in the application of these compounds, have implicitly studied the active ingredients, adjuvants, and impurities. In some cases, a shortcoming of epidemiology is that it is not possible to separate out the effects of individual chemicals that make up a workplace exposure. However, this is an advantage with respect to impurities and adjuvants because the epidemiology findings *de facto* consider all of the above. The epidemiology studies reviewed were, therefore, considered relevant to both 2,4-D exposure and exposure to adjuvants and impurities.

The various ester and amine formulations of 2,4-D contain very small quantities (0.1 to approximately 5%) of adjuvants and inert ingredients such as ethoxylated castor oil, EDTA salts, lignosulfamate salts, antifoamers (*e.g.*, dimethylpolysiloxane), and emulsifiers (Page, personal communication). Inert compounds or adjuvants added to pesticide formulations are regulated under CFR 40 § 180.1001 and are exempted from tolerances when used in accordance with good agricultural practice. For this reason, these compounds are not discussed further.

There have been some concerns with respect to possible contamination of 2,4-D formulations with substances which may potentiate adverse effects. Most noted are the polychlorinated dibenzo-*p*-dioxins (PCDDs), particularly, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (T_4CDD). In addition, some attention has been given to possible nitrosamine contamination of 2,4-D.

The formulations of 2,4-D manufactured and sold in the U.S. contain very few PCDD contaminants. Earlier herbicide mixtures of 2,4,5-T and 2,4-D contained 2,3,7,8-T_4CDD. With the identification of 2,3,7,8-T_4CDD in 2,4,5-T formulations, study of the possible dioxin content of 2,4-D alone was undertaken. No evidence of T_4CDD was reported in any 2,4-D formulations that did not also contain 2,4,5-T (Woolson *et al.*, 1972; Cochrane *et al.*, 1981; 1982a,b).

Some 2,4-D formulations may contain very low levels of other much less potent PCDDs. To regulate this, the Canadian government set a limit of 10 ppb total PCDD in 2,4-D formulations. In the U.S., no such limit has been set. Cochrane *et al.* (1981; 1982a,b) analyzed more than 200 samples of 2,4-D esters and amines by gas chromatography/mass spectrometry and identified 2,7- or 2,8-dichlorodibenzo-*p*-dioxin (D_2CDD), 1,3,7- or 1,3,8-trichlorodibenzo-*p*-dioxin (T_3CDD), and 1,3,6,8-T_4CDD. All, but a very few samples tested, contained concentrations well below the 10 ppb limit.

Most toxicological data pertaining to dioxin are based on the most potent form, 2,3,7,8-T_4CDD. The toxicity of the majority of the other congeners, particularly those identified in 2,4-D at very low levels, is considered minor in comparison.

Nitrosamine, particularly *N*-nitrosodimethylamine (NDMA), levels also have been studied in earlier 2,4-D formulations (Cohen *et al.*, 1978; WHO, 1984; Reid, 1984). Most samples tested contained less than 1 ppm NDMA (Cohen *et al.*, 1978; Reid, 1984) with a very few samples containing greater than 5 ppm (Reid, 1984). Newer 2,4-D formulations would no longer be expected to contain nitrosamines, formed from nitrates used in preserving metal storage containers, since storage conditions have changed from metal containers to plastic or epoxy-lined containers (Hammond, personal communication). In addition, the EPA no longer requires analysis for N-nitroso contaminants in formulations such as 2,4-D so long as nitrates, nitrites, or other nitrosating agents are not used in the product or its packaging (Lindsay, 1989).

The adjuvants, impurities, and inert ingredients possibly detected in various 2,4-D formulations are of no toxicological concern, since they either fall under the EPA regulations or are virtually not detectable in present-day formulations.

Patterns of human exposure

Since 2,4-D is one of the most common herbicides, studies have shown that human exposure to it occurs with a wide spectrum of uses, ranging from domestic to commercial applications. Possible routes of exposure include inhalation, ingestion, and dermal contact. Respiratory exposure to 2,4-D is very low (less than 2%) (Grover *et al.*, 1986). Residual levels of 2,4-D in foodstuffs or drinking water have been found to be, for the most part, non-detectable or only detected in trace amounts (Duggan and Lipscomb, 1969; Duggan and Corneliussen, 1972; Gartrell *et al.*, 1985). Furthermore, in a study with wild berries, there was an obvious trend in reduction of 2,4-D detected from very small amounts to non-detectable or trace amounts between the years 1979 and 1981 (Frank *et al.*, 1983). The greatest exposure to 2,4-D occurs through direct dermal contact during use of the product.

With the growing regard for worker safety, greater emphasis has been placed on protective clothing in the workplace. In the case of pesticide application, or specifically 2,4-D application, protective clothing was not required until recent years, and therefore, most of the past epidemiological studies conducted do not reflect a growing trend towards the use of protective apparel when applying herbicides. With the new proposed labeling directions on products containing 2,4-D, workers are required to wear protective clothing consisting of eye protection (*i.e.,* face shield or safety glasses), chemical resistant gloves, long-sleeved shirt, long pants, socks, and shoes. It is also recommended that workers wash hands, face, and arms with soap and water as soon as possible after 2,4-D and before eating, drinking, or smoking. In addition, all contaminated clothing should be removed and washed separately after each use.

The main pathways for human exposure to 2,4-D involve the workplace, and in cases of home use, application of the product (Lavy and Mattice, 1984; Frank *et al.*, 1985; Grover *et al.*, 1986; Yeary, 1986; Lavy *et al.*, 1987; Harris *et al.*, 1992). Accidental exposures due to drift and improper application have been documented (Lavy and Mattice, 1984; Harris *et al.*, 1992). Five main exposure

groups have been identified for evaluation in this assessment: home and garden users, bystanders, farm workers, commercial applicators, and forestry workers. Each group represents a different exposure scenario and is outlined below. In all groups, the exposure can be described as repeated subchronic in duration, since the utilization of 2,4-D is mostly seasonal and short-term in nature.

Home and Garden Users

Exposures to home and garden users tend to be of short duration (approximately one day). Although comprehensive data are not available on use patterns, it can be assumed based on label directions that home and garden users generally would not use 2,4-D formulations more than one to four times per year. In the proposed label directions, 2,4-D use would be limited to two applications per year (Shearer, personal communication).

2,4-D applied by the home and garden user is normally in a liquid or granular form in combination with other herbicides such as dicamba. The liquid concentrate is mixed with water for spraying, whereas the granular form (often in the form of a fertilizer) is used in its purchased form.

Bystanders

In cases of home and garden use and accidental exposure to commercial/forestry application, exposures to bystanders can be assumed to be no more than a few times per year since these exposures tend to be of an accidental nature, and the likelihood for a bystander having multiple exposures would appear to be low; however, some bystanders who are regularly present during commercial or forestry application of 2,4-D (*e.g.*, supervisors), may have higher levels of exposures.

Farm Workers

In 1992, the Canadian Centre for Toxicology prepared a report on past and existing crop spraying practices (CCT, 1992). From the 1940s onward, methods of pesticide application developed from dusters to boom sprayers. Although spraying and application practices have not changed a great deal since the 1940s (CCT, 1992), the use of more protective equipment (*e.g.,* cab or covered tractors) has increased. Eighty to 95% of herbicides are applied by ground spraying and 5 to 10% (less than 5% of

2,4-D specifically) are applied through aerial spraying (CCT, 1992).

Over 90% of farmers spray their own crops (CCT, 1992; Grover, personal communication). The average prairie farmer spends approximately two to ten hours per day, two to four days per year (with a maximum of ten days per year) spraying herbicide formulations (Grover, personal communication). Farmers generally use 2,4-D in combination with other herbicides such as dicamba, MCPA, and mecoprop (CCT, 1992; Olsen, 1992) and almost always prepare the mixture with water (Grover, personal communication). In one of the epidemiology studies (Zahm *et al.*, 1990), it was reported through telephone interviews that some farmers in Nebraska may apply or mix 2,4-D more than 21 days per year; however, this made up only a very small proportion (less than 7%) of the farmers interviewed. The majority (37 to 45%) of the farmers used 2,4-D one to five days per year and to a lesser extent (26 to 28%) six to 20 days per year. The remaining farmers interviewed did not give an estimated use level. Similarly, farmers in North Dakota worked an average of three (range one to 14) days per year (Nash *et al.*, 1982).

Commercial Applicators

The typical commercial applicator (*i.e.*, weed control) spends approximately 80 to 100 days per year spraying with formulations containing 2,4-D and 40 to 50 days per year spraying with other herbicides. In one day, a worker spends on average two to three hours actually spraying herbicides. In general, 30% of a typical work day is spent spraying the herbicide. The rest of the time includes driving between job sites and administrative work (Yeary, personal communication). Commercial applicators spraying wheat crops by air in Washington state worked an average of five (range three to 16) hours per day, 45 (range 14 to 60) days per year (Nash *et al.*, 1982). Ontario Hydro herbicide applicators have been reported to spend an average of approximately five hours per day spraying (Libich, 1981).

In commercial applications, 2,4-D is almost always used in combination with other herbicides such as dicamba or MCPP and the herbicide concentrate is prepared with water for spraying (Yeary, personal communication).

Forestry Workers

The level of exposure to a herbicide varies with the job description of the forestry worker who may be part of a ground or aerial crew. Comprehensive data on exposure scenarios for each job type were not available; however, some concept of exposure variation in relation to job type can be gleaned from a report by Shipp *et al.* (1986). In the report, some description was given pertaining to the requirements of the Department of Natural Resources (DNR-Washington state) for the aerial application of herbicides. The spray crew described consisted of a pilot, loader (batchman/mixer), and mechanic. For the most part, the pilot remained in the helicopter, but may have helped unclog congested nozzles occasionally. The loader had the highest potential of exposure since this individual mixed the herbicide concentrate, loaded it into the helicopter, and often remained in the spray area during herbicide application. The loader often used a cloth rag to wipe up any spills. The mechanic often helped the loader, but was mainly responsible for manipulating the shut-off valve during loading, stopping leaking boom nozzles, and cleaning the helicopter bubble. In addition to the spray crew, three bystanders were often present and consisted of the local government representative, the district manager, and the area forester. These bystanders occasionally assisted in the spray operations by moving empty drum containers.

According to the DNR, aerial spray operations occur three months out of the year. Most aerial spraying is conducted at approximately 7.5 m above the tree tops with a swath width of approximately 14 m (Shipp *et al.*, 1986).

Ground crews consist of backpack workers, injection bar workers, hypohatchet workers, and hack-and-squirt workers (Lavy *et al.*, 1987). In the Lavy *et al.* (1987) study, backpack workers had a much higher exposure to the herbicide mixture, since the workers would walk back through the sprayed foliage. Injection bar workers were not as exposed to the herbicide mixture, since any spray back from the bar would strike an area on the worker that was well covered with protective clothing. The exposure encountered by the hypohatchet workers was between that of the backpack workers and the injection bar workers. Hack-and-squirt workers showed a higher exposure than expected due to leaky squirt bottles, but it was still much lower than that of the backpack workers. It should be noted that in this study, the backpack workers were spraying a two-fold dilution of the active ingredient as compared to the other workers.

The average forestry worker sprays 2,4-D approximately 20 days per year. Backpack workers tend to spray less often at approximately 10 days per year (Lavy, personal communication). Hours of spraying

per day ranged from approximately six to eight hours per day (Frank *et al.*, 1985; Knopp and Glass, 1991).

For most forestry applications, 2,4-D is mixed with other herbicides such as dichlorprop and picloram (Lavy, personal communication). The herbicide concentrate is usually mixed with water (95% of the time), but on occasion may be mixed with petroleum distillates (5% of the time) (Lavy, personal communication).

Quantitative measured exposures in various user groups

In evaluating the overall scientific information on the potential health risks of 2,4-D, knowledge of exposure pathways is critical. Realistic assumptions about exposure are necessary first steps in arriving at realistic appraisals of potential health risks based on data from both toxicology studies and epidemiology studies.

A literature review was conducted that identified and evaluated studies examining potential human exposure to 2,4-D. The purpose of this review was to compile all relevant data in order to estimate the potential exposure to 2,4-D for various exposed groups. In compiling the data, emphasis was placed on the identification of: the receptor group (*i.e.*, individuals exposed to 2,4-D); the estimated internal dose of 2,4-D to which the receptors were exposed (expressed as µg/person and µg/kg body weight/day); and, the number of days per year the receptors were typically exposed.

Whenever possible, exposure was expressed as the internal dose in units of "µg/kg body weight/day". Exposure was preferentially expressed as internal dose since this represented the total absorbed dose from all exposure routes (*i.e.*, the internal dose represented the sum of exposure from the oral, dermal, and inhalation routes). In many studies, the investigators estimated the internal dose based on urinary excretion of 2,4-D following exposure and these values were used to estimate exposure. Since nearly 100% of the 2,4-D absorbed into the body is rapidly excreted into the urine (Kohli *et al.*, 1974; Feldmann and Maibach, 1974; Sauerhoff *et al.*, 1977), urinary excretion is an accurate method to estimate the internal dose. In a number of studies, the authors reported urinary excretion data but did not express the data in µg/person or µg/kg body weight. In these circumstances, the internal dose was calculated using the urinary excretion data available with receptor data (such as body weight) that was

provided specifically by the investigators or considered representative of the population.

In many of the studies reviewed, the individuals studied did not apply 2,4-D while wearing appropriate protective clothing to minimize exposure. In these circumstances, exposures were relatively high but are not representative of current practices or appropriate use conditions. For example, commercial applicators who did not take appropriate protective action had average estimated exposures ranging from 2 to 160 μg/kg body weight/day (Draper and Street, 1982; Nash *et al.*, 1982) and forestry workers who did not take appropriate protective action had exposures ranging from 0.86 to 129 μg/kg body weight/day (Kolmodin-Hedman and Erne, 1980; Lavy *et al.*, 1987).

To estimate exposures in this report, data were only used from studies which documented 2,4-D use under appropriate conditions of protection. For the purposes of this exposure assessment, appropriate conditions of protection were defined according to label directions. More specifically, protective application was defined as the wearing of a minimum of: boots made of rubber or other materials of low chemical permeability, rubber gloves, and coveralls. The potential exposure to 2,4-D associated with each of the receptor groups applying 2,4-D under appropriate protective conditions is discussed below.

Home and Garden User Exposure

In a recent home and garden study by Harris *et al.* (1992), approximately 90% of the subjects showed no detectable level of 2,4-D from the use of granular or liquid 2,4-D (see Table 1, Appendix A). The remaining subjects presumably were exposed to 2,4-D by removal of protective clothing for short periods during the application procedure. If these individuals are taken into consideration, the average internal dose received by home and garden users was 0.13 and 0.14 μg/kg body weight/day for liquid and granular use, respectively, as shown in Table 1. The arithmetic mean of the average estimated internal dose to home and garden users was 0.14 μg/kg body weight/day.

Bystander Exposure

Levels of 2,4-D were measured in bystanders of home and garden use (Harris *et al.*, 1992) and of commercial applicator use (Lavy and Mattice, 1984). Home and garden bystanders showed no

detectable levels of exposure to 2,4-D when exposed twice per year for one day whereas bystanders observing aerial application crews received an average estimated internal exposure of 0.09 μg/kg body weight/day when exposed for one day, as shown in Table 1.

Farm Worker Exposure

No studies were identified which allowed estimation of the internal dose that farm workers wearing appropriate protective apparel received from spraying their fields. One study was identified in which farmers applied 2,4-D to their fields but did not take protective action (Grover *et al.*, 1986). In this study farmers generally did not wear gloves, respirators, or other special protective equipment. From this study, eight farmers were estimated to receive an average internal dose of 2,4-D of 5.78 μg/kg body weight/spray operation. One to seven spray operations were spread over a 17-day spray season.

Commercial Applicator Exposure

Only one study was identified which examined the internal dose received by various groups of commercial applicators (*i.e.*, lawn care specialists) wearing appropriate protective apparel (Yeary, 1986). This study indicated that the groups of commercial applicators had average estimated internal doses ranging from 0.35 to 6.3 μg/kg body weight/day, as shown in Table 1. The arithmetic mean of the average estimated internal dose of commercial applicators was 2.75 μg/kg body weight/day.

Forestry Worker Exposure

Three studies were identified in the literature which examined exposure to forestry workers wearing protective apparel (Lavy and Mattice, 1984; Frank *et al.*, 1985; Lavy *et al.*, 1987). In classifying forestry workers, all aerial application crews were included as well as individuals working on the ground. The following exposure estimates are summarized from Table 1.

1. Aerial application crews: mixers/loaders/batchmen had estimated internal doses ranging from 0.53 to 4.52 μg/kg body weight/day; pilots had an average estimated internal dose of 8.54 μg/kg body weight/day; one mixer/balloon man had an estimated internal dose of 4.95 μg/kg body weight/day; one balloon man had an estimated internal dose of 3.91 μg/kg body

weight/day; in two studies, supervisors had average estimated internal doses ranging from 0.01 to 0.22 μg/kg body weight/day; and, mechanics had an average estimated internal dose of 3.01 μg/kg body weight/day.

2. Ground crews: backpack workers had estimated internal doses ranging from 30 to 244 μg/kg body weight/day with an average of 98 μg/kg body weight/day; injection bar workers had estimated internal doses ranging from not detectable to 12.1 μg/kg body weight/day with an average of 4.3 μg/kg body weight/day; hypohatchet workers had estimated internal doses ranging from 0.7 to 104 μg/kg body weight/day with an average of 40 μg/kg body weight/day; and, hack-and-squirt workers had estimated internal doses ranging from not detectable to 60 μg/kg body weight/day with an average of 12.2 μg/kg body weight/day.

In the Lavy *et al.* (1987) study, the doses received by the ground crew workers appeared relatively high, especially since the workers were supposed to represent workers wearing protective clothing. Upon consultation with the study author, it was determined that, for the most part, the workers did not wear protective clothing beyond boots and gloves. The author also indicated that during the period of the study, the weather was very hot and often the workers would remove their shirts, especially the backpack workers. The hypohatchet and hack-and-squirt workers had leaky bottles and often spilled the herbicide mixture on themselves (Lavy, personal communication).

Summary of human exposure to 2,4-D

Table 1 is a summary table which provides the ranges of typical expected exposure for each of the receptor groups. A detailed compilation of these data is provided in Appendix A. From the information presented in this table, it is clear that home and garden users, as well as bystanders, have the lowest potential exposure to 2,4-D. Farm workers and commercial applicators showed a slightly higher potential exposure whereas forestry workers, particularly backpack workers, have the highest potential exposure.

METABOLISM AND GENOTOXICITY

Summary

Once an exposure has been sustained by an individual, the amount of dose delivered to the critical tissue is an important determinant of whether or not a health risk exists. The delivered dose is a function of the absorption and metabolism of the compound in the host.

The absorption, distribution and excretion data on 2,4-D, its salts and esters, demonstrate consistency among species in terms of rapid absorption from the gastrointestinal tract, rapid and essentially complete urinary excretion and lack of accumulation potential. Absorption is slower and less complete when 2,4-D is applied dermally with the rate of absorption varying with different chemical forms and formulations, species and site of application in an inconsistent manner. In humans, the percent of the dimethylamine salt absorbed through the skin was 58%, while the percent of the isooctyl ester absorbed was 6%. The relative amounts of these absorbed by the monkey were reversed, illustrating the difficulty in formulating a general conclusion regarding dermal absorption rates. Studies have consistently shown that absorption of 2,4-D through the skin is the major route of exposure to humans, resulting in measurable body burden of the compound. Inhalation accounts for less than 2% of the total body burden.

Once in the body, 2,4-D has a very short biological half-life, estimated to be between 10 and 36 hours. In the absence of sustained exposure, data suggest that nearly 100% of 2,4-D body burden is cleared within two to four days of exposure.

2,4-D is a simple organic acid with no structural features known to be associated with chemical reactivity or genotoxicity. The available data indicate that 2,4-D is not metabolized to reactive intermediates in mammalian systems, including humans. The compound is predominantly excreted in the urine as parent compound. This is important because nearly all classical chemical carcinogens exert their cancer-causing impact through metabolic activation products.

Overall, the metabolism data suggest that 2,4-D does not have the characteristics usually associated with substances that produce toxic effects through critical reactive intermediates. This is consistent

with the apparent lack of genotoxic potential indicated by the results of numerous genotoxicity assays. 2,4-D does not accumulate in any tissue, or cause significant target organ effects at dose levels below those causing saturation of renal clearance, as such it does not have the characteristics of a compound which could induce cell or tissue damage leading to regenerative hyperproliferation and subsequent tumorigenesis.

Absorption, distribution and excretion

Absorption

2,4-D is absorbed very quickly from the gastrointestinal tract of the rat with 30% of a 1 mg/kg body weight dose being absorbed within 10 minutes of dosing, and 75% absorbed within two hours (Pelletier *et al.*, 1989). The rate of absorption from the gastrointestinal tract is dependent on dose, with a much higher percentage (7% *versus* 0.4%) of an oral dose remaining in the stomach of rats 24 hours after a dose of 240 mg/kg body weight than 12 hours after a dose of 3 mg/kg body weight (Khanna and Fang, 1966). Gastrointestinal absorption was also slightly faster after a 0.4 mg/kg body weight oral dose than after a 1 mg/kg body weight oral dose in rats (Pelletier *et al.*, 1989). Peak plasma concentrations of 2,4-D were reached within eight hours of oral dosing in a number of species (Erne, 1966a; Khanna and Fang, 1966). In rats given oral doses of 0.4 or 1 mg/kg body weight, peak plasma levels were reached within 10 and 20 minutes respectively (Pelletier *et al.*, 1989). In humans given a dose of 5 mg 2,4-D/kg body weight, absorption was rapid and complete, with peak plasma levels occurring after four hours in one study (Sauerhoff *et al.*, 1977), and within seven to 24 hours in a similar study (Kohli *et al.*, 1974). Hydrolysis and subsequent absorption of the 2,4-D esters may occur more slowly than for the acid or salt forms, as evidenced by the longer blood half-life of the butyl ester in rats (Erne, 1966a). The longer half-life of the butyl ester is not attributable to differences in excretion rate among the various 2,4-D forms, since butyl ester administered by subcutaneous injection to rats at the same dose as used by Erne (1966a) was 90-100% excreted within 48 hours (Shulze *et al.*, 1985), indicating an excretion rate of the ester that is similar to that of the acid and salt forms given orally (Khanna and Fang, 1966; Knopp and Schiller, 1992; Pelletier *et al.*, 1989). In rats given an oral dose of 130 mg of the 2-ethylhexyl ester, only the acid form was detected in blood and urine after 72 hours, with 95% of the dose having been excreted in males and 84% in females by this time (Frantz and Kropscott, in press). The absorption data for 2,4-D indicate that it is rapidly and completely absorbed

from the gastrointestinal tract, with no major differences in absorption rate among animal species.

Dermal absorption of 2,4-D appears to be the major route in humans exposed under occupational conditions, with estimates that approximately 90% of total exposure is through this route (Feldman and Maibach, 1974). Dermal absorption is rapid as evidenced by the finding that 2,4-D was detectable in the urine of humans four hours after exposure (Feldman and Maibach, 1974). Absorption through the human forearm was calculated to be 6.37% in 120 hours (Fisher *et al.*, 1985) based on measured urinary excretion of 5.79% of the dose within 120 hours (Feldmann and Maibach, 1974). Dermal absorption of 2,4-D varies with a number of factors including species, site of application, form of 2,4-D, vehicle (Moody *et al.*, 1990; 1991), and method of pretreatment of application site (Pelletier *et al.*, 1990). In a study of the dermal absorption of 2,4-D acid, dimethylamine salt, and isooctyl ester in rabbits, rats, monkeys and humans, differences in the amounts absorbed over the course of seven days were observed among species, but these were not consistent across the various forms and formulations of 2,4-D (Moody *et al.*, 1990; 1991). For example, the fraction of the dose of the amine absorbed from the forehead was 31% in monkeys and 58% in humans, while the pattern was reversed for the isooctyl ester with 56% being absorbed in monkeys and 6% in humans. In this study, the skin was washed 24 hours after application of the 2,4-D. The monkey forehead was found to be more permeable than the monkey forearm to the acid, amine and ester forms of 2,4-D. In the animal model most commonly used for the study of 2,4-D absorption, the shaved rat back, only the amine was tested. The fraction absorbed over the course of seven days was 20%, lower than the 31% and 58% absorbed by monkey and human forehead, respectively (Moody *et al.*, 1990; 1991). In other dermal absorption studies using rats, the percent absorption was 18 to 21% of a 10 mg/kg body weight dose of the dimethylamine salt over 24 hours (Pelletier *et al.*, 1990), 10 to 15% of a 2.6 mg/kg body weight dose of the sodium salt or a 1.9 mg/kg body weight dose of the dimethylamine salt over 69 hours (Knopp and Schiller, 1992) and 85% of a 5 mg/kg body weight dose of the butylether ester over 120 hours (Smith *et al.*, 1980). When denuded rat skin was washed seven hours after application of a 10 mg/kg body weight dose of the dimethylamine salt, 63% of the dose was removed, while 17% remained in the skin at the dosing site an hour later and 13% remained 72 hours post-dosing (Pelletier *et al.*, 1989). In a similar experiment, 60% of the dose was removed by washing after eight hours, while 40% was removed if washing was delayed until 24 hours after dosing (Pelletier *et al.*, 1990). Interestingly, a second cleansing at 23 hours after a previous cleansing at seven hours post-dosing resulted in higher blood levels of 2,4-D at 24 hours than did a single cleansing at seven hours, suggesting mobilization and further absorption of 2,4-

D caused by cleansing (Pelletier *et al.*, 1990). In mice treated dermally with a 1 mg/kg body weight dose of 2,4-D acid, the extent of absorption was 10% by eight hours, 20% by 24 hours and 40% by 48 hours (Grissom *et al.*, 1987). The data on dermal absorption of 2,4-D indicate variability with chemical form, vehicle, species, and site of application. In humans, absorption over seven days without washing may be as high as 58% for the dimethylamine salt; however, evidence from studies with rats suggests that this value would be much lower if washing occurred within eight hours of exposure.

Although dermal exposure is the major route of occupational exposure to 2,4-D (IARC, 1987; Veterans Administration, 1981a,b; WHO, 1984), humans may be exposed to 2,4-D through inhalation to some degree. There have been no controlled human or animal studies of inhalation exposure; however, the rapid excretion of 2,4-D following occupational exposure indicates rapid absorption by the combination of dermal and inhalation routes (Kolmodin-Hedman and Erne, 1980; Frank *et al.*, 1985). In one study with farmers, the authors estimated respiratory exposure to account for less than 2% of total exposure (Grover *et al.*, 1986).

Distribution

Due to its high degree of water solubility, 2,4-D is widely distributed throughout the body, but does not accumulate in any tissue. Having a pKa of 3.0, 2,4-D exists predominantly in the ionized form at physiological pH and would not readily cross lipid membranes. Entry into tissues and across the blood/brain barrier involves active ion transport systems (Berndt and Koshier, 1973; Kim and O'Tuama, 1981; Pritchard, 1980). Tissue levels in rats given single oral doses peaked at six to eight hours after a dose of 3 or 240 mg/kg body weight (Khanna and Fang, 1966) and at 10 and 20 minutes following doses of 0.4 and 1 mg/kg body weight, respectively (Pelletier *et al.*, 1989). In a variety of species studied, 2,4-D was detected in liver, kidney, and lung, and at lower levels in the brain after oral dosing (Erne, 1966a). Similarly, in sheep and cattle given 2,4-D at levels of 200, 1000, or 3000 ppm in feed for 28 days, concentrations were highest in liver and kidney at the end of the dosing period and dropped significantly within a week, indicating a lack of accumulation potential (Clark *et al.*, 1975). Brain levels of 2,4-D in rats given a single dose of 7 mg/kg body weight by injection were very low (Tyynela *et al.*, 1990). In rats, levels in all tissues peaked at six hours and dropped rapidly over 24 hours (Erne, 1966a). The average volume of distribution of 2,4-D among six volunteers who ingested a 5 mg/kg body weight dose was 100 ml/kg body weight (Kohli *et al.*, 1974), within the range of 20 to

300 ml/kg body weight reported for three volunteers consuming a similar dose in another study (Sauerhoff *et al.*, 1977). These low volumes of distribution indicate lack of extensive tissue distribution of 2,4-D in humans (Kohli *et al.*, 1974; Sauerhoff *et al.*, 1977). Levels of 2,4-D in the brain and cerebrospinal fluid of rats increased relative to plasma levels at intoxicating doses, suggesting that 2,4-D intoxication increases the influx of 2,4-D into the brain, or decreases efflux out of the brain (Elo and Ylitalo, 1977; 1979). In rats given an oral dose of 300 mg 2,4-D/kg body weight, severe intoxication was induced and the brain:blood ratio of radioactivity from a subsequent 7 mg/kg body weight dose of labeled 2,4-D was eight-fold higher than the brain:blood ratio associated with a 7 mg/kg body weight dose given to rats not previously intoxicated (Tyynela *et al.*, 1990). The rise in brain levels of 2,4-D relative to plasma levels appears to become very steep between doses of 100 and 250 mg/kg body weight (Elo and Ytlitalo, 1979). Further studies on the blood/brain transport of 2,4-D have indicated that efflux from the brain occurs via an active organic acid transport system (Kim and O'Tuama, 1981; Pritchard, 1980), and that organic acid transport out of the brain is inhibited by high concentrations of 2,4-D (Kim *et al.*, 1983; Pritchard, 1980; Tyynela *et al.*, 1990; Ylitalo *et al.*, 1990). In addition, the accumulation of 2,4-D in the brain at toxic doses is likely facilitated by increased influx of 2,4-D through the compromised blood/brain barrier (Elo *et al.*, 1988; Hervonen *et al.*, 1982; Tyynela *et al.*, 1990). Vascular damage to various areas of the brain was observed in rats given 2,4-D at doses of 300 and 600 mg/kg body weight, but not at 150 mg/kg body weight (Elo *et al.*, 1988).

A factor involved in determining the distribution of 2,4-D is its capacity to bind to serum proteins (Erne, 1966b). A correlation exists between *in vitro* protein binding affinity and blood:tissue concentration ratios of 2,4-D, suggesting a relationship between protein binding and tissue distribution (Fang and Lindstrom, 1980). At low plasma levels of 2,4-D in goats, nearly all the 2,4-D was bound to albumin, while at higher levels only 65% was bound, suggesting saturation of albumin binding (Orberg, 1980). *In vitro*, the binding affinity and extent of binding of 2,4-D to serum albumin was similar using purified protein from rats or humans (Fang and Lindstrom, 1980). This, along with similarities between rats and humans with respect to absorption and excretion rates, indicates that the rat is a suitable model for the extrapolation of pharmacokinetic data to humans. Saturation of plasma protein binding at intoxicating doses of 2,4-D is probably partly responsible for the increased brain:blood ratio of 2,4-D observed in rats, although inhibition of organic anion transport, as discussed above, appears to play a more significant role (Tyynela *et al.*, 1990; Ylitalo *et al.*, 1990).

Placental transfer of 2,4-D has been observed in laboratory animals. A small degree of fetal exposure was observed by autoradiography in the fetuses of late-stage pregnant mice given a single intravenous dose of 0.05 mg 2,4-D. No radioactivity associated with 2,4-D was present 24 hours after dosing, demonstrating rapid elimination from the fetus (Lindquist and Ullberg, 1971). In pregnant rats given an oral dose of 0.05 mg/kg body weight ^{14}C-2,4-D, radioactivity was detected in the uterus, placenta, fetus and intrauterine fluid at levels of 2.7, 3.5, 4.7 and 4.9% of the administered dose, respectively (Fedorova and Belova, 1974). In pigs fed 2,4-D at 500 ppm in the diet throughout pregnancy, 2,4-D was found in liver, kidney, and lungs of fetuses, as well as in the placenta (Erne, 1966a).

Excretion

2,4-D is excreted rapidly and almost exclusively in urine in all species studied (Erne, 1966a; Moody *et al.*, 1990; 1991). In rats given oral doses of 30 mg/kg body weight or lower, 90% or more of the administered dose was excreted in urine within 24 hours (Khanna and Fang, 1966; Knopp and Schiller, 1992; Pelletier *et al.*, 1989). Urinary excretion was also the major route in rats administered 2,4-D dermally (Knopp and Schiller, 1992; Moody *et al.*, 1990; 1991; Pelletier *et al.*, 1989). A tracer dose of radiolabeled 2,4-D was 100% excreted in the urine of humans after five days (Feldman and Maibach, 1974). In five human volunteers given single oral doses of 5 mg 2,4-D/kg body weight, the amount of the dose excreted in urine over six days ranged from 87 to 100% (Sauerhoff *et al.*, 1977). In a similar study using six subjects, the mean cumulative excretion over 96 hours was 76.5$\pm$8.4% (Kohli *et al.*, 1974).

The urinary excretion rate of 2,4-D is highly dependent on dose, with the percent of the dose excreted in urine becoming lower at high doses. At oral doses of 1 to 10 mg (approximately 3 to 30 mg/kg body weight), urinary excretion in rats accounted for 93 to 96% of the administered dose within 48 hours, while at doses of approximately 60 to 300 mg/kg body weight, the fraction of the dose excreted within 24 hours decreased linearly with increasing dose (Khanna and Fang, 1966). 2,4-D is excreted by the kidneys via renal tubular secretion, an active, saturable process (Berndt and Koschier, 1973; Gehring and Betso, 1978; Orberg, 1980). In rats given oral doses of 10, 25, 50, 100, or 150 mg/kg body weight, kidney concentrations of 2,4-D were 6.6, 5.5, 3.7, 2.1, and 1.6 times plasma concentrations, respectively, providing evidence of saturation of excretion at a dose of 50 mg/kg body weight and above (Gorzinski *et al.*, 1987). This is in agreement with the results of Khanna and Fang (1966), which

indicated that the percent of an oral dose excreted in urine within 24 hours was greater than 90% at doses of 5 to 30 mg/kg body weight, dropped below 90% at 60 mg/kg body weight, and became even lower at higher doses.

Perspiration appears to be another significant route of excretion in humans occupationally exposed to 2,4-D. Three agricultural workers received dermal exposure to the hands over the course of two hours, and 2,4-D in T-shirts and urine was monitored for two weeks following exposure (Sell *et al.*, 1982). 2,4-D was measurable in perspiration via T-shirt extracts for two weeks and was measurable in urine for five days. The ratio of 2,4-D in perspiration compared to urine ranged from 1:10 to 1:1 among the three workers.

A minor route of elimination appears to be via milk in lactating animals. Radioactivity was detected in the gastrointestinal tract of pups nursed by rats given an oral dose of 2,4-D of 100 mg/kg body weight right after delivery. Radioactivity associated with 2,4-D was detected in seven-day old pups, with maximum levels detected in two- or three-day old pups (Fedorova and Belova, 1974).

Pharmacokinetics

As discussed in Metabolism of 2,4-D, peak plasma levels of 2,4-D occur soon after oral or dermal dosing. Plasma half-lives following an oral dose of 100 mg/kg body weight ranged from 3.5 to 12 hours in a variety of species given different forms of 2,4-D (Erne, 1966a). In rats given an oral dose of 240 mg/kg body weight, plasma and tissue half-lives were in the range three-3.5 hours, while in rats given a lower dose of 3 mg/kg body weight, plasma and tissue half-lives were 0.5 to 0.8 hours (Khanna and Fang, 1966). This illustrates the dependence of clearance rates on dose. Non-linear pharmacokinetic were also observed in rats given oral doses of 10, 50, or 150 mg or intravenous doses of 5 or 90 mg 2,4-D, with plasma levels increasing and urinary excretion decreasing relative to dose at doses over 50 mg (Smith *et al.*, 1980). In a detailed pharmacokinetics study using rats, plasma clearance following oral doses of 0.4 or 1 mg/kg body weight was found to follow a three compartment model, with half-lives of 0.2 to 1.1, 1.3 to 7.1, and 15.5 to 101.5 hours for the three compartments, respectively (Pelletier *et al.*, 1989). Excretion was almost exclusively in urine and followed first order kinetics. In humans given 2,4-D in a single oral dose of 5 mg/kg body weight, plasma clearance followed first order kinetics in two out of three subjects and followed biphasic

kinetics in one subject (Sauerhoff *et al.*, 1977). The plasma half-lives were 7.29 and 11.2 hours in the two subjects showing first order plasma clearance, and the half-lives in the other subject were 4.25 hours and 16.2 hours for the two phases, respectively. Despite the apparent differences in clearance pharmacokinetics among the three subjects, the overall clearance rates were not markedly different among them. Urinary excretion followed first order kinetics in all three subjects. Urinary excretion half-lives were determined in five subjects, and ranged from 10.2 to 28.4 hours (Sauerhoff *et al.*, 1977). The average plasma clearance half-life among six subjects given oral doses of 5 mg 2,4-D /kg body weight was 33$\pm$3 hours, assuming first order clearance (Kohli *et al.*, 1974). These laboratory findings are consistent with the urinary excretion half-life of 18 hours determined in a forestry worker who exhibited the highest amount of 2,4-D excretion among two groups exposed over the course of 11 or 18 days (Frank *et al.*, 1985). Slightly longer half-lives, in the range of 35-48 hours were reported for agricultural sprayers (Nash *et al.*, 1982).

Dermal administration of 2,4-D to rats results in a different pharmacokinetic profile from that observed in rats dosed orally (Pelletier *et al.*, 1989). Between two and eight hours after dermal administration the blood levels reached a plateau, after which they declined rapidly. In contrast, plasma levels peaked more quickly and then decreased rapidly following an oral dose. Plasma clearance of a dermal dose followed biphasic kinetics beginning eight hours post-dosing, with half-lives for various tissues ranging from 0.6 to 2.3 hours for the first phase and 25.7 to 29 hours for the second phase. Cumulative urinary excretion of 2,4-D increased at a slower rate after dermal exposure than after oral exposure, due to the slower absorption rate and the presence of a reservoir of 2,4-D on the skin.

Metabolism of 2,4-D

The salts and esters of 2,4-D undergo acid and/or enzymatic hydrolysis in body fluids and tissues to form 2,4-D acid. In pigs given repeated oral doses of 50 mg/kg body weight, and in rats given a single oral dose of the butyl ester, only trace amounts of the ester form were present in blood (Erne, 1966b). Only 2,4-D acid was detected in the blood or urine of rats given a 130 mg/kg body weight dose of the 2-ethylhexyl ester (Frantz and Kropscott, in press). Similarly, in rats administered a 100 mg/kg body weight dose of the butyl ester by subcutaneous injection, no parent compound was detectable in urine, although less than 2% of the administered dose was excreted as a sidechain metabolite of the butyl ester (Schulze *et al.*, 1985). The presence of acid hydrolyzable conjugates of 2,4-D in the urine has

been reported in laboratory animals and humans. This conjugate represented from 0 to 18% of the 2,4-D excreted in urine by pigs given repeated oral doses of 50 mg/kg body weight, or consuming diets containing 500 ppm 2,4-D for five months (Erne, 1966b). 2,4-D conjugate accounted for less than 5% of the 2,4-D in the plasma of the pigs receiving dietary 2,4-D (Erne, 1966b). In rats given oral doses of 2,4-D, a small amount of an unidentified metabolite amounting to 0.25% of recovered radioactivity was excreted in urine (Khanna and Fang, 1966). Conjugates excreted by rats given an oral dose of 200 mg 2,4-D/kg body weight were identified as glycine and taurine conjugates, each of which accounted for 1.4% of the dose (Grunow and Bohme, 1974). A small amount of glycine conjugate was also detected in liver perfusates *in vitro* (Kelley and Vessey, 1987). An acid hydrolysable conjugate was detected in the urine of four out of five human subjects after ingestion of 5 mg 2,4-D/kg body weight. The percent of the administered dose excreted as the conjugate ranged from 4.8 to 27.1%, representing a large degree of inter-individual variation (Sauerhoff *et al.*, 1977). In a similar study using six human volunteers, no metabolic products of 2,4-D were detected in urine by gas chromatography (Kohli *et al.*, 1974). No conjugated metabolites were detected in the urine of rats given a subcutaneous injection of the butyl ester at a dose of 100 mg/kg body weight. This lack of conjugation may be due to limitation of the availability of a free carboxyl group as a result of the rate of metabolic hydrolysis of the ester to the acid (Schulze *et al.*, 1985). Acid extraction of tissue from sheep or cattle fed diets containing 300, 1000, or 3000 ppm 2,4-D acid did not increase 2,4-D recovery (Clark *et al.*, 1975), indicating the absence of conjugated metabolites. Despite the sensitive analytical tecniques used in many of these studies, the only metabolites detected have been conjugated detoxification products, with no evidence of the production of a reactive intermediate.

Genotoxicity studies

Genotoxicity studies provide a means of indirectly detecting the presence of metabolically formed reactive intermediates. The results from a wide variety of *in vitro* and *in vivo* genotoxicity bioassays indicate that 2,4-D has little, if any, genotoxic potential. These findings are consistent with the lack of evidence for the presence of reactive intermediates obtained in metabolism studies, and are consistent with the lack of structural features of 2,4-D which are known to be associated with biological or chemical reactivity. A number of these *in vitro* and *in vivo* mutagenicity/genotoxicity bioassays are summarized in Tables 1 and 2 in Appendix B.

The results of most of the bacterial mutagenicity tests including *Salmonella typhimurium* and *Escherichia coli*, have been essentially negative both with and without an exogenous source of metabolic activation (see Table 1 in Appendix B). Testing of yeast has been reported to induce mitotic gene conversion and recombination (Zetterberg *et al.*, 1977; Zetterberg, 1978). However, this effect appeared to be highly pH-dependent, occurring only at pH 4.3 and 4.5 (Zetterberg, 1978). Tests conducted with and without the presence of rat S9 (post-mitochondrial liver homogenate) at other pH values gave negative results (Zetterberg *et al.*, 1977; Simmon *et al.*, 1977; Waters *et al.*, 1980). In mammalian cell lines, in which unscheduled DNA repair and sister chromatid exchange (SCE) bioassays were conducted, either negative or only weakly positive results have been reported (Styles, 1977; Waters *et al.*, 1980; Galloway *et al.*, 1987; Clausen *et al.*, 1990; Jacobi and Witte, 1991). The marginally positive results often occurred only at toxic doses (Korte and Jalal, 1982; Clausen *et al.*, 1990). Based on a weight-of-evidence approach, the *in vitro* mutagenicity/genotoxicity data suggest that 2,4-D has no significant genotoxic potential. A similar conclusion was reached in a previous review of the genotoxic potential of 2,4-D (CCT, 1987). The lack of genotoxicity in *in vitro* bacterial and mammalian test systems which have included an exogenous source of metabolic activation provides evidence that 2,4-D is not metabolized to potentially reactive intermediates.

In plant cells, 2,4-D has been demonstrated to induce chromosomal aberrations (Khalatkar and Bhargava, 1985; Sidorov *et al.*, 1988; Pavlica *et al.*, 1991). However, as discussed in the CCT report (1987), since 2,4-D was developed to be highly active in and toxic to plants, studies on the genotoxicity of 2,4-D in plants seem inappropriate for the determination of genotoxic potential in humans.

The *in vivo* genotoxicity of 2,4-D also has been extensively reviewed (CCT, 1987). Several additional *in vivo* studies, including SCE and chromosomal aberration tests of lymphocytes from exposed human populations and corresponding tests in experimental animals, have been published which were not reviewed in the CCT (1987) report. A summary of the available *in vivo* genotoxicity studies which were reviewed by CCT (1987) or published afterwards is presented in Table 2 in Appendix B. The new results do not alter the conclusion expressed in the previous review (CCT, 1987). The weight of evidence indicates that 2,4-D is not genotoxic in *in vivo* mammalian studies. Due to the importance of *in vivo* studies, however, they are briefly described below.

Linnainmaa (1983a) and Linnainmaa and Vainio (1983) reported that there was no significant effect of occupational exposure to 2,4-D on SCE frequency. Furthermore, there was no apparent dose-response between the reported urinary 2,4-D concentrations and the number of SCEs observed in the peripheral lymphocytes. SCEs were elevated in both the exposed and control groups of workers who were also smokers. A similar observation was also reported by Mustonen *et al.* (1986), in which exposure to 2,4-D had no apparent effect on SCE frequency since the incidence of SCE was elevated in smokers of both the control and exposed group.

Certain reports in humans exposed to 2,4-D (Yoder *et al.*, 1973; Crossen *et al.*, 1978; Kaye *et al.*, 1985), which have shown marginal or positive activity in lymphocytes, appear to be flawed because of multiple chemical exposures of the subjects, poor controlling of other confounding factors, low numbers of cells which were analyzed, and chromosomal aberration and SCE frequencies which showed considerable variation. In light of this, and given that there have been several studies of human lymphocytes exposed *in vivo* which indicate that 2,4-D exposure, as well as concomitant exposure to other chemicals, has no effect on chromosomal aberration or SCE frequency (Hogstedt and Westerlund, 1980; Mulcahy, 1980; Linnainmaa 1983a,b, 1984; Mustonen *et al.*, 1986, 1989), there seems to be no conclusive evidence to suggest that 2,4-D is clastogenic in humans.

In a study of 26 Idaho herbicide sprayers, Yoder *et al.* (1973) reported a significant increase in the incidence of breaks (0.07 to 1.81) in the lymphocyte assays conducted at mid-season when compared to the assay results from the off-season. The off-season results, however, were very low compared to results from unexposed controls (0.31). This variability may be due to the fact that only 25 cells per person were assayed at any given test period. It is difficult to associate the increased SCE in the mid-season tests of herbicide workers solely with 2,4-D since the workers also reported exposures to amitrole, atrazine and to a lesser extent, eleven other formulations. In addition, there was no apparent attempt to control what are now known to be confounding effects of smoking on the incidence rates of chromosomal abnormalities.

In another study of 57 New Zealand herbicide workers, Crossen *et al.* (1978) reported that the rate of SCE in herbicide sprayers who wore no protection was significantly elevated, while those wearing some or complete protection had SCE incidence rates that were not significantly greater than the controls. Since the unprotected worker group was exposed to 2,4,5-T and many other chemicals in

addition to 2,4-D, the positive result reported for SCE in unprotected herbicide sprayers cannot be ascribed solely to 2,4-D.

Human exposure to Agent Orange also has been reported to be associated with an increased incidence of chromosome damage (Kaye *et al.*, 1985). However, in this study, as in many of the other *in vivo* human studies, there was no controlling for smoking, age, sex or any other socio-economic factors. Mixed exposure also occurred in the group of Vietnam veterans studied by Kaye *et al.* (1985) since Agent Orange contains significant quantities of 2,4,5-T and lesser quantities of 2,3,7,8-T_4CDD as a contaminant.

The results of several SCE studies, using the peripheral lymphocytes of rats and the bone marrow of mice and Chinese hamsters, indicate that 2,4-D exposure in animals does not result in significant clastogenic activity (Lamb *et al.*, 1981a,b; Linnainmaa, 1984; Mustonen *et al.*, 1986, 1989). In addition, two mouse micronucleus bioassays have indicated that exposure to 2,4-D at doses to 100 mg/kg via intraperitoneal injection (Jenssen and Renberg (1976) or to ¼ of the dermal LD_{50}[2] (Schop *et al.*, 1990) does not result in the induction of micronuclei.

A few researchers have reported results for *in vivo* genotoxicity testing of 2,4-D that would appear not to be in accordance with the weight of the experimental data (Pilinskaya, 1974; Turkula and Jalal, 1987; Adhikari and Grover, 1988; Konstantinova and Shevelo, 1984). Pilinskaya (1974) reported an increase in the number of chromosomal aberrations in the bone marrow of mice treated with doses of approximately 100 and 300 mg/kg body weight. At lower doses (*i.e.*, 10 and 50 mg/kg body weight), no effect on chromosomal aberration was observed. The results from the Pilinskaya (1974) study can be viewed as equivocal given the high dose levels administered. The negative control was not specified as a vehicle control, and the test results fell within the range of values for the negative controls. There were no positive control rodents, nor was the experiment reproduced.

The genotoxic effects reported in rats exposed to 75, 100, and 150 mg/kg for 24 hours (Turkula and Jalal, 1987) were only clearly apparent when chromosome gaps were included in the analysis. The number of chromosome breaks that was statistically significant in one of three replicate experiments could not be reproduced. No effects on chromosomal aberrations, even in the one replicate which

[2] dose at which 50% of the experimental population dies

showed some effect, were noted in the rats exposed to 125, 200, or 300 mg/kg for either four or 24 hours. Adhikari and Grover (1988) have reported increased chromosomal damage in rat bone marrow as a result of the exposure of rats to two consecutive daily doses of either 35 or 70 mg/kg administered via intraperitoneal injection. In addition to the lack of information on the test material that was injected, studies using intraperitoneal injection are not considered to be reflective of *in vivo* studies or relevant to human exposure (Ashby, 1985.)

Konstantinova and Shevelo (1984) reported increased numbers of chromatid bridges and higher coefficients of mitotic phases in the bone marrow of albino rats after exposures to 1 mg 2,4-D butyl ether six times/week for 6.5 months. In the absence of actual experimental data, however, the significance of the results reported is questionable.

Results indicative of *in vivo* genotoxicity were also reported for 2,4-D by Schop *et al.* (1990) in the mouse hair follicle nuclear aberration assay; however, this particular test has not been validated for the purposes of quantitating genotoxic potential. In addition, certain other non-carcinogenic agents have been observed to induce a positive response in this assay (*e.g.*, DMSO, Schop *et al.*, 1990) as well as in other anomaly assays. Similarly, Seiler (1979) reported that a single 200 mg/kg body weight dose of 2,4-D induced inhibition of testicular DNA synthesis in mice, an assay later understood to be unreliable and not subsequently used.

In *Drosophila melanogaster*, the great majority of the results have been negative (Vogel and Chandler, 1973, 1974; Magnusson *et al.*, 1977; Woodruff *et al.*, 1983; Zimmering *et al.*, 1985). Somatic mutations, induced by 2,4-D exposure, have been observed only in unstable strains of *Drosophila melanogaster* (Magnusson *et al.*, 1977; Rasmuson and Svahlin, 1978).

In summary, 2,4-D has been demonstrated to have no, or only very limited, genotoxic/mutagenic potential *in vitro*. In *in vivo* testing, most researchers have reported that 2,4-D is inactive in tests which have examined the peripheral lymphocytes of humans exposed to 2,4-D and other compounds for the presence of chromosomal aberrations and SCEs. The marginally positive results reported in a few of the human monitoring studies can not be related specifically to 2,4-D exposure since there were confounding exposures to a wide variety of other chemicals and there was inadequate controlling for confounding factors (*e.g.*, age, sex, race, and smoking status). Results of the *in vivo* bioassays in

experimental animals tend to support the conclusion that 2,4-D is non-mutagenic and non-genotoxic. A few researchers have reported results that appear to disagree with the majority of the other published reports. Following thorough review of these reports and based on a weight-of-evidence approach that includes data from the *in vitro* and *in vivo* genotoxicity studies conducted according to the most accepted and validated procedures (Williams, 1989), it may be concluded that 2,4-D is not genotoxic in mammals.

TOXICOLOGICAL, EPIDEMIOLOGICAL AND OTHER DATA RELATED TO CARCINOGENICITY

Summary

The chronic bioassays in rats and mice on 2,4-D are of sufficient quality to draw conclusions regarding cause and effect if adverse effects had been noted. The doses used in these studies were considerably higher than those to which humans may be exposed when protective clothing is worn, exceeding those of the highest documented exposed forestry workers by a factor of nearly 5000. The doses in the definitive animal cancer bioassays were 1, 5, 15, and 45 mg/kg body weight/day with the highest dose selected to fall near the threshold for renal tubular secretion, identified as 50 mg/kg body weight/day in metabolic studies. The results from the chronic animal studies do not indicate that 2,4-D is an animal carcinogen. While a significantly increased incidence of brain astrocytomas in male rats at the highest dose of 45 mg/kg body weight/day was reported in one study, the tumors were not characteristic of those induced by chemical carcinogens and the weight of evidence suggests that these tumors were incidental and not likely to be associated with 2,4-D treatment. The lack of evidence of tumorigenesis from animal bioassays is consistent with the chemical characteristics, the metabolic data and the lack of a plausible mechanism of tumorigenesis for 2,4-D. Overall the weight of evidence from animal studies provides no reason to expect that 2,4-D would be a human carcinogen.

With regard to human epidemiology, study methods employed to date have been unable to provide definitive assessments of exposure to 2,4-D. Among these studies with speculative and indirect indices of exposure, the findings have been inconsistent, suggesting that evidence for a causal association between 2,4-D and cancer in general or of any particular site is weak. Where exposure data that appear to be somewhat specific to 2,4-D exist (such as in worker cohort studies), no association

between 2,4-D and human cancer has been convincingly documented.

Case-control studies suggesting positive associations between high frequency use of herbicides and NHL, the most persistent of the cancer hypotheses, are equivocal in that exposure to 2,4-D was inferred from self-reporting or next-of-kin reporting and not directly measured, point estimates of risk are low, and findings are based on very small numbers of cases in the various exposure subgroups. The exposures reported in these studies were likely to be very low and usually mixed chemical exposures, with those in the highest exposure groups receiving estimated annual doses of only 120 μg 2,4-D/kg body weight. Only an extremely potent carcinogen, more potent than any carcinogen yet identified, would be capable of inducing tumorigenesis at such a low dose, and as such would be expected to produce tumors in animals treated at doses orders of magnitude higher. This has not proven to be the case.

The cohort studies have better documentation of exposure because this information is determined from employment records rather than from recall after the diagnosis of disease, but not all of the phenoxy acid exposures were specifically 2,4-D. Although in some cohorts a slightly increased risk of lymphopoietic cancer or STS cannot be ruled out, the studies are inconsistent as to the type of cancer showing an increase and as to the exact type of phenoxy acid to which the cohort was exposed. Four of the cohorts reporting increased lymphopoietic cancer or STS provide only limited information because of the small sample sizes and resultant low power. The only positive results in the three large cohort studies conflict, each showing an increase in only one of the three cancers of concern, and a different cancer in each cohort: significantly increased HD among farmers and forestry workers exposed primarily to 2,4,5-T, insignificantly increased STS among workers exposed to multiple phenoxy herbicides and chlorophenols, and slightly significantly increased NHL associated with number of acres sprayed with herbicides.

The hypothesis that 2,4-D causes NHL in humans is not supported by observed target tissue effects in animals. Animal studies of immune system toxicity and carcinogenicity also do not add support to the hypothesis. In addition, the epidemiology of NHL suggests that viruses and immune system modulation are risk factors, and these potential confounders have not been adequately controlled in the existing epidemiology studies of herbicide use.

Overview of Human Epidemiology Studies

Since the hypothesis that phenoxy acid herbicides might be associated with rare forms of cancer was first raised in the 1970s, more than ninety reports of epidemiological studies that provide data relevant to the 2,4-D and cancer hypothesis have appeared in the scientific literature. Brief synopses of these studies can be found in Table 2 and more detail on the cohort studies is presented in Table 3.

Observations gleaned in reviewing the totality of these epidemiology studies include the following:

1) Qualitative and ecological correlational data from studies of farmers, forestry workers, and other similar groups of potential users of herbicides are prevalent among these data. The earliest of these types of studies provided the basis for raising hypotheses about possible cancer risks from exposure to 2,4-D; however, subsequent studies have continued to suffer from many of the same methodological shortcomings of the hypothesis-generating studies, particularly with regard to exposure assessment. In effect, many of the later studies do not provide a basis for rigorously testing the 2,4-D cancer hypotheses and moving the scientific understanding of 2,4-D forward.

2) Few of the published studies were able to verify either the fact of exposure to phenoxy acid herbicides or the level of exposure. The majority of the published studies contained speculation regarding exposure to phenoxy acid herbicides and 2,4-D in particular, based on occupational descriptions such as farming and forestry. These occupations encompass exposures to a wide variety of chemical, physical, and biological agents in addition to phenoxy acid herbicides. Absent specific exposure information, testing of the 2,4-D cancer hypothesis from the perspective of independence of effects and dose-response has remained questionable, at best, in the majority of the published studies.

3) Cohort studies in which exposure to phenoxy acid herbicides, and 2,4-D in particular, could be reasonably assumed based on job descriptions, have not confirmed the original hypotheses addressing an association between 2,4-D and either HD, STS, NHL, although Bond *et al.* (1988) provided some support for the NHL hypothesis by finding two NHL in a cohort of workers involved in 2,4-D manufacturing, formulating or packaging. The other cohort studies

that have suggested slight increased risks of lymphopoietic cancer or STS have conflicted not only in types of cancer but also in specific phenoxy acids encountered and confounding factors reported (Lynge, 1985; Wiklund and Holm, 1986; Wiklund *et al*., 1987; 1988a,b; 1989; Wigle *et al*., 1990; Coggon *et al*., 1991; Saracci *et al*., 1991; Hansen *et al.*, 1992). The only positive results in the three large cohort studies conflict, each showing an increase in only one of the three cancers of concern, and a different cancer in each cohort: significantly increased HD among farmers and forestry workers exposed primarily to 2,4,5-T (Wiklund and Holm, 1986; Wiklund *et al*., 1987; 1988a), insignificantly increased STS among workers exposed to multiple phenoxy herbicides and chlorophenols (Saracci *et al*., 1991), and slightly significantly increased NHL associated with number of acres sprayed with herbicides (Wigle *et al*., 1990).

4) The most persistent hypothesis regarding 2,4-D and cancer bears on a possible association with NHL. Case-control studies of farmers in Kansas and Nebraska have provided the basis for the hypothesis that use of 2,4-D for 21 or more days per year in farming carries an increased risk of NHL (Hoar *et al*., 1986; Zahm *et al*., 1990). Our review indicates that this hypothesis is not strongly supported by those data.

First, the estimated risk increases observed in these studies were derived to a large extent from next-of-kin responses to questionnaires regarding exposure to herbicides in general. There is reasonable doubt about whether the next of kin would be knowledgeable of a subject's daily weed control practices or able to recall with precision such practices followed by the subject 15 to 20 years earlier. Second, from a practical perspective, it appears unlikely that an appreciable number of farmers would have the need to use a herbicide like 2,4-D for more than twenty days per year. These two potential sources of misclassification along the exposure gradient reported in these studies could be an important consideration. We suspect that such misclassification could be differential, due to the likelihood of cases and their next-of-kin spending much time searching for a possible explanation for their illness. The results in both the Kansas and Nebraska studies are of marginal statistical significance, with the Kansas finding dependent upon seven cases of NHL and the Nebraska finding upon three cases reporting use of herbicides for more than 20 days per year. Misclassification of the exposure variable for only a few cases of NHL would cause the finding of increased risk associated with 21 days or more per year of use to disappear, or lose statistical significance. Third, the epidemiology of

NHL suggests that viruses and immune system modulation are risk factors, and these potential confounders have not been adequately controlled in the existing epidemiology studies of herbicide use (Gibbs *et al*., 1987; Hardell, 1981; Mills, 1988; Pearce *et al*., 1986a; Saito *et al*., 1989; Snyder *et al*., 1980).

5) Overall, the epidemiology studies support the concept that broadly defined occupational groups such as farmers and forestry workers may be at increased risk for certain types of cancers, particularly lymphopoietic cancer. However, more refined studies that were capable of addressing the 2,4-D cancer hypothesis with the benefit of more reliable exposure data do not prove the thesis that 2,4-D is a cause of cancer.

Discussion of key epidemiology studies

The Hardell Studies

In 1978, Hardell first reported on a case-control study of malignant mesenchymal tumors in patients with exposure to phenoxyacetic acids or chlorophenols in Sweden (Hardell and Sandstrom, 1978; 1979). With information on use patterns of herbicides and chlorophenols obtained from questionnaires, the investigators observed that 36.5% of the cases and 9.2% of the controls recalled exposures to these compounds. The investigators suggested a six-fold increase in risk for STS; however, they cautioned that the effect could be due to dioxin exposures. The median latent period was 15 years.

The first study to raise the phenoxy acid herbicide and cancer hypothesis, it was subjected to rigorous peer review within the scientific community, resulting in the expression of concerns about the methodology employed in gathering information through questionnaires. Prior to the publication of Hardell's study in 1978, the case-control questionnaire methodology had not been used extensively to study environmental exposures such as pesticides, in large part because there were questions regarding the ability of such an approach to identify useful exposure information. To enhance the ability to identify past exposures to herbicides and other related chemicals, Hardell and his co-workers employed multiple layers of probes by interviewers. Although interviewers were supposedly blinded as to the case or control status of the respondent, the method proved difficult to standardize and many important

methodological and interpretational questions were raised by the approach.

In the years following, Hardell and his co-workers employed the same methodology in other case-control studies, repeating their STS hypothesis and suggesting associations between phenoxy acid herbicides, chlorophenols or dioxins and malignant lymphoma, HD, and nasal and pharyngeal cancer (Hardell, 1979; Eriksson *et al.*, 1981; 1990; Hardell *et al.*, 1981; 1982; Hardell and Axelson, 1982). Reviewers suggested that the methodology was biased and that a positive association nearly always resulted when it was employed (Bond *et al.*, 1989).

Hardell (1981) attempted to address the criticisms that his methods would find an increased risk for any exposure studied, by publishing a study of colon cancer where the same methodology was employed but no risk increase was identified. The concern about Hardell's methodology, however, has persisted in the scientific community. Reviewers such as Sir Richard Doll have commented that Hardell's work had so many opportunities for bias to have been introduced, such as searching for exposure after determining outcome, and that Hardell seemed to have published such an amount of exaggerated or non-supportable statements, that his work should no longer be cited as scientific evidence (Doll, 1988). In addition, a large cohort study conducted in Sweden after the publication of Hardell's work, and encompassing the same workers studied by Hardell, failed to show the associations between exposure to phenoxy acid-related compounds and STS, NHL, or HD, except possibly HD in forestry workers exposed primarily to 2,4,5-T (Wiklund and Holm, 1986; Wiklund *et al.*, 1987; 1988a). It would be expected that the results would be replicated in the larger cohort study if the original case-control findings were valid.

Cohort and Case-Control Studies Testing Hardell's Hypotheses

Methodological shortcomings notwithstanding, the Hardell work spawned a number of cohort and case-control studies aimed at testing his hypotheses. Besides Hardell's work, we reviewed 47 reports of cohort studies, and 42 reports of case-control studies, issued from 1978 to 1992, that contained information relevant to the hypotheses raised by Hardell (see Table 2). These studies considered manufacturing workers, forestry workers, farmers, and other groups with occupational environments logically suggesting potential exposures to phenoxy acid herbicides in general and 2,4-D in particular. In the majority of these studies, exposure to phenoxy acid herbicides in general or to 2,4-D specifically

was speculative or inferred based on overall descriptions of the workplaces or environments being studied. The case-control studies attempted to assess historical exposure based on questionnaire recall. Because determination of exposure and controlling for confounding in many of these studies was not more rigorous than in the earlier studies, the value of the majority of these studies in testing the hypothesis that phenoxy acid herbicides cause cancer is limited.

A number of studies, however, were carried out on populations with a high likelihood of exposure to phenoxy acid herbicides.

Two PMR[3] studies looked at STS, HD and NHL mortality in occupations with exposure to phenoxy herbicides and chlorophenols (Milham, 1982; Gallagher and Threlfall, 1984). Since PMR studies examine proportions of deaths, interpreting them requires caution because they assume that a given exposure affects only the death rate for the category under consideration. In Washington State, deaths in all occupations with exposure to phenoxy herbicides and chlorophenols were not elevated for HD or NHL, and deaths from STS were slightly but not statistically significantly elevated (Milham, 1982). In British Columbia, deaths in occupations with exposure to phenoxy herbicides and chlorophenols were slightly elevated for HD, NHL and STS, but the only statistically significant elevations were for HD in the occupations primarily exposed to chlorophenols (Gallagher and Threlfall, 1984).

Barthel (1981) studied 1658 German men exposed to pesticides for at least five years between 1948 and 1977. Exposures included: the fungicides Cupral, Zineb and Maneb; the insecticides arsenic, DDT, HCH, parathion-methyl, toxaphene, dimethoate, trichlorphon, piperonyl butoxide and carbaryl; the herbicides DNOC, 2,4-D, MCPA, 2,4,5-T, Dalapon, Simazine, amitrole, atrazine, chlorpropham, prometryn, desmetryn, pyrazon, chloral hydrate Bi 3411; and maleic hydrazide. Bronchial carcinoma was the only cancer that was significantly higher (doubled) in the cohort than in the general population. Smoking was not controlled in the analyses, although a survey of 163 members of the cohort and 163 members of the general population did not indicate any significant differences in smoking or tobacco consumption habits. The author suggested that at least some of the excess lung cancer might be due to the exposure to arsenic.

[3]proportional mortality or morbidity ratio

Riihimaki *et al.* (1982; 1983) studied the mortality experience of a cohort of 1,926 men who had sprayed 2,4-D and 2,4,5-T during 1955 and 1971, with prospective follow-up from 1972 to 1980. The total phenoxy acid exposure was generally rather low because the duration of work had mostly been less than two months. For the period from 1972 to 1976, mortality from all natural causes in the cohort was only 54% of the expected value (based on age-specific rates for the general population), and 81% of the expected value in the succeeding four-year period. In the assessment of cancer, mortality allowance was made for 10 and 15 year periods of latency between the first exposure and the start of the recording of vital status during the follow-up. No increase in cancer mortality was detected, and the distribution of cancer types was unremarkable. No cases of death from lymphomas or STS were found. An assessment of mortality in the subpopulation with the longest duration of exposure did not demonstrate higher risk than the whole cohort.

Environmental Health Associates (1983) studied 1535 current and former Chevron Chemical Company employees involved in the manufacture and formulation of agricultural chemicals, including 2,4-D, consumer products and fertilizers. Overall mortality for the cohort was 16% lower than expected. Cause-specific mortality deficits were observed in diseases of the circulatory system, non-malignant respiratory disease, and diseases of the digestive system. Mortality from cancer of all sites and respiratory cancer was as expected. There was a non-significant mortality excess in cancer of the digestive system, but the excess did not come from cancer of any particular organ. A non-significant mortality excess was also found in kidney cancer, although a detailed review of the work histories of the two observed kidney cancer cases did not reveal any epidemiologic relationship between the observed excess and the work environment. Mortality from brain cancer and lymphopoietic cancer was as expected. The manufacturing subcohort had results similar to the overall cohort. The authors concluded that there was no evidence of any work-related mortality problems within this cohort, but the follow-up was comparatively short, with more than one-third of the cohort having a latent period less than 10 years.

Blair *et al.* (1983) evaluated the mortality of 3827 white males who were licensed pesticide applicators in Florida. The cohort included those certified in the categories of "termites," "household pests," "rodents," "fumigation" and "lawn," so the men were exposed to numerous chemicals, including 2,4-D. Deaths from leukemia, brain cancer and lung cancer were elevated, although none statistically significant. Smoking was not controlled. Mortality from STS and lymphomas was not elevated.

Lynge (1985) conducted a follow-up study of cancer incidence among workers involved in the manufacture of phenoxy herbicides in Denmark. The authors stated that the purpose of this cohort study was to shed further light on the potential carcinogenic effect suggested by Eriksson and Hardell of the 2,4-dichlorophenol and 4-chloro-ortho-cresol based phenoxy herbicides, unlikely to be contaminated with 2,3,7,8-T_4CDD (Eriksson *et al.*, 1981). The investigators included all persons employed in the manufacture of phenoxy herbicides in Denmark before 1982. The predominant product was 4-chloro-2-methyl-phenoxyacetic acid (MCPA), but 2,4-D was also produced. Only a very limited amount of 2,4,5-T was processed in one of the two factories included in the study. Registration of the cohort was based on company records, supplemented with data from a public pension scheme from 1964 onwards. Ninety-nine percent of registered employees were followed up. Cancer cases were identified by linkage with the National Cancer Register. Totals of 3,390 males and 1,069 females were included in the study. In the analysis, special attention was given to STS and malignant lymphomas (ML). Five cases of STS were observed among male employees compared to 1.84 expected cases (RR[4] 2.72, CI[5] 0.88-6.34). Seven cases of ML were observed among male employees compared to 5.37 expected. While this slight increased risk is similar in magnitude to the risks reported in the NCI case-control studies that will be discussed later, there is actually little consistency between the reports because these Danish workers were exposed primarily to MCPA and their increased risk was in ML, while the NCI studies were of farmers exposed to 2,4-D and other herbicides, who exhibited increased risks of NHL alone. Most importantly, none of the Danish cases occurred in the phenoxy acid department, while six of the seven occurred in the pigment department. The total cancer risk among persons employed in manufacture and packaging of phenoxy herbicides was equivalent to the cancer risks in the Danish population. Among males thus employed 11 lung cancer cases were observed in contrast to 5.33 expected (RR 2.06, CI 1.03-3.69). Cigarette smoking, however, was not taken into account.

It should be noted that each of the above cohort studies were of small sample size and therefore of limited power. Doublings of risk in those studies could have been masked because of the limited power. On the other hand, the consistency in finding little evidence of increased cancer risk across

[4]relative risk

[5]confidence interval

these studies is informative.

Wiklund and colleagues studied the risk of STS following possible exposure to phenoxy acid herbicides in 354,620 Swedish men who were employed in agriculture or forestry according to a national census in 1960 (Wiklund and Holm, 1986; Wiklund *et al.*, 1987). This cohort was further divided into six subcohorts, with different assumed exposures to phenoxy acid herbicides. The most commonly used phenoxy acids in Sweden were MCPA in agriculture and 2,4,5-T in forestry, but 2,4-D has also been used in agriculture and forestry. The reference cohort encompassed 1,725,845 Swedish men employed in other industries. All persons were followed up in the cancer-environment register during the period from 1961 to 1979. A total of 331 cases of STS was observed in the study cohort and there were 1,508 cases in the reference group (RR 0.9, CI 0.8-1.0). No subcohort of agricultural or forestry workers showed any significantly increased risk, nor was there any significant difference in risk between the subcohorts. Despite the greatly increased use of phenoxy acid herbicides from 1947 to 1970, no time-related increase in the risk of STS was found in the total cohort or in any of the subcohorts. This same study population showed no increase in NHL and HD was significantly elevated only among farmers and forestry workers who were exposed primarily to 2,4,5-T (Wiklund *et al.*, 1988a).

In a subsequent study, Wiklund and colleagues studied the risk of STS, HD and NHL among Swedish licensed pesticide applicators (Wiklund *et al.*, 1988b; 1989). In the cohort of 20,245 Swedish pesticide applicators, 72% were estimated to have been exposed to phenoxy acid herbicides. The most commonly used phenoxy acid was MCPA, but 2,4-D was also used. A total of seven patients with STS, 15 with HD and 27 with NHL were observed compared to 7.7, 10.2, and 25.3 expected, respectively. The RRs were 0.91 (CI 0.37-1.88) for STS, 1.47 (CI 0.82-2.42) for HD, and 1.07 (CI 0.70-1.55) for NHL. A marginally statistically significant increased HD risk (RR 2.18, CI 1.00-4.14) for persons born 1935 or later was found, but there was no trend with date of birth and risk was not significantly elevated in the entire cohort. No increased risks for STS and NHL were found.

Bond *et al.* (1988) studied cause-specific mortality among employees engaged in the manufacture, formulation, or packaging of 2,4-D and related salts. The follow-up was to the end of 1982 for 878 chemical workers potentially exposed to 2,4-D at any time between 1945 and 1983. Observed mortality was compared with expected levels based on adjusted rates for U.S. white men and for other

male employees from this manufacturing location who were not exposed to 2,4-D. Certain SMR[6]s were statistically significantly elevated: cancer of other and unspecified sites in the entire cohort (306, CI 101-729), all lymphopoietic cancer in the 2,4-D plant (312, statistically significant at $\propto = 0.05$) and cancer of ill-defined sites in the 2,4-D plant (388, statistically significant at $\propto = 0.05$). Analyses by duration of exposure and cumulative dose showed no patterns suggestive of a causal association between 2,4-D exposure and any particular cause of death, although the small number of deaths makes these findings less conclusive. This study by Bond was a well-conducted analysis of a small cohort of workers definitely exposed to 2,4-D, with a latency period adequate for most types of cancer. The study, however, had only modest power to detect risk increases for specific cancer sites.

Bloemen (1990) updated the Bond *et al*. (1988) study. With 100% follow-up, observed deaths were compared both with those expected based on adjusted rates for the U.S. white population and also with the mortality experience of other workers in the manufacturing location who were not exposed to 2,4-D. The longer period of follow-up modestly increased the power of the study to detect increases in risk compared to the original work by Bond *et al*. (1988). Because of suggestions in some case-control studies of an association between NHL and prior exposure to herbicides, including 2,4-D, special attention was given to this cause of death. Two deaths from NHL had been observed in the original study. No new cases were found during the updated period, causing the elevated SMR for all lymphopoietic cancer in the 2,4-D plant to lose statistical significance. Analysis by duration of exposure, cumulative dose and latency period did not show patterns suggestive of a causal relationship between 2,4-D or its derivatives and any particular cause of death, although the small number of deaths makes these findings less conclusive. Cancer of "other and unspecified sites" had an SMR of 351 ($p < 0.05$) in the 2,4-D plant; however, the author did not offer an interpretation suggesting this to be chemical-related.

Wigle *et al*. (1990) studied the almost 70,000 male farmers in Saskatchewan and found no excess mortality for any cause of death, including NHL. The authors did report a significant correlation between NHL and acres sprayed with herbicides (predominantly 2,4-D) and a slightly significant increased risk of NHL corresponding to the highest number of acres sprayed (RR 2.2, CI 1.0-4.6). They also reported, however, a significant correlation between NHL and purchases of fuel and oil for

[6]standardized mortality or morbidity ratio

farm purposes, as well as a significant increased risk of NHL associated with the highest value of fuel purchased (RR 2.3, CI 1.1-4.7). The exposure data were self-reported.

Coggon *et al.* (1991) studied mortality and incidence of cancer at four factories making phenoxy herbicides in Europe as part of the International Agency for Research on Cancer (IARC) collaborative study of workers exposed to these compounds in their production or use. Four British cohorts of chemical manufacturers which have been recruited to the survey were included. All four cohorts were exposed to 2,4-D, but also to MCPA, 2,4,5-T and other phenoxy acids. They comprise a total of 2239 men employed from 1963 through 1985. These subjects were traced to the end of December 1987 through the National Health Service Central Register and the National Insurance Index, and their mortality compared with that in the national population. Two deaths from NHL were identified, compared with 0.87 expected (SMR 229, CI 28-827). Both deaths occurred more than 10 years after first exposure to phenoxy compounds. One further NHL was registered in a living subject with probable exposure to phenoxy compounds. No cases of STS or HD were recorded. A non-significant excess of lung cancer, with 19 deaths observed where 14.2 were expected (SMR 134, CI 81-210) is probably attributable to chance or a confounding effect of smoking. In only one cohort, there was increased mortality from circulatory disease (34 deaths observed where 20.4 were expected, not statistically significant). A nested case-control study did not point to any occupational cause for this excess.

Green (1991) examined the mortality of 1222 men who were routinely exposed to herbicides, including 2,4-D and other phenoxy acids, at a public electrical utility in Ontario for six months or more between 1950 and 1982. No overall excess mortality mortality was seen in the cohort and no deaths were seen due to STS or NHL. The only statistically significant SMR was for suicide (210, $p = 0.04$).

Saracci *et al*. (1991) analyzed the International Register of Workers Exposed to Phenoxy Herbicides and their Contaminants established by the International Agency for Research on Cancer and the U.S. National Institute of Environmental Health Sciences. The cohort was compiled from ten countries and contained 18,910 production workers or sprayers exposed to phenoxy herbicides and chlorophenols. The cohorts of Lynge (1985), Coggon *et al*. (1991) and Green (1991), discussed above, were included in the larger Saracci *et al*. cohort. No excess mortality was seen in all-cause mortality, all-cancer mortality, or lymphoma mortality. A statistically insignificant SMR of 196 (CI 53-502) was seen for

STS, based on four deaths that occurred ten to 19 years after first exposure (SMR 606, CI 165-1552), three of which deaths were in sprayers (SMR 882, CI 182-2579). These workers were exposed to 2,4-D, 2,4,5-T, MCPA, 2-(4-chloro-2-methyl-phenoxy)-propanoic acid (MCPP), 4-(4-chloro-2-methyl-phenoxy)-butanoic acid (MCPB), phenoxybutyric acid, 2-(2,4-dichlorophenoxy)-propanoic acid, 4-(2,4-dichlorophenoxy)-butanoic acid, 2-(2,4,5-trichlorophenoxy)-propanoic acid, 2,4-dichlorophenol, 2,4,5-trichlorophenol, 2,4,6-trichlorophenol, 2,3,4,6-tetrachlorophenol, pentachlorophenol, and T_4CDD. Which, if any, of these chemicals caused the reported STS increase could not be determined.

Hansen *et al*. (1992) recently reported on the incidence of cancer among 4015 Danish gardeners. Males showed significantly increased STS (SMR 526, CI 109-1538) and chronic lymphatic leukemia (SMR 275, CI 101-599) and insignificantly increased NHL (SMR 173, CI 63-376); females showed no STS or chronic lymphatic leukemia but insignificantly increased NHL (SMR 364, CI 44-1314). Most of the male gardeners worked outdoors, where they were exposed to 2,4-D, 2,4,5-T, MCPA, amitrole, DDT and other insecticides, and fungicides. Almost all of the female gardeners worked in greenhouses, where they were exposed to DDT, chlordane, lindane, organophosphorous compounds, nicotine, and other insecticides and fungicides. These multiple chemical exposures make it hard to attribute the increased cancer incidences to 2,4-D, especially since the NHL occurred both among males, who were exposed to 2,4-D, and females, who were not.

With the exception of Wigle *et al*. (1990), in which exposure was self-reported, exposure in these cohorts was determined through employment records rather than through recall after diagnosis of disease. Not all of the phenoxy acid exposures were specifically 2,4-D, however, and other confounding factors existed. These cohort studies are not persuasive in showing a link between exposure to 2,4-D and cancer, although in some a slightly increased risk of lymphopoietic cancer or STS cannot be ruled out (Lynge, 1985; Wiklund and Holm, 1986; Wiklund *et al*., 1987; 1988a,b; 1989; Bond *et al*., 1988; Wigle *et al*., 1990; Coggon *et al*., 1991; Saracci *et al*., 1991; Hansen *et al*., 1992). The studies are inconsistent, however, as to the type of cancer showing an increase and as to the exact type of phenoxy acid to which the cohort was exposed. Four of the cohorts reporting increased lymphopoietic cancer or STS provide only limited information because of the small sample sizes and resultant low power (Lynge, 1985; Bond *et al*., 1988; Coggon *et al*., 1991; Hansen *et al*., 1992). The only positive results in the three large cohort studies conflict, each showing an increase in only one of the three cancers of concern, and a different cancer in each cohort: significantly increased

HD among farmers and forestry workers exposed primarily to 2,4,5-T (Wiklund and Holm, 1986; Wiklund *et al*., 1987;1988a), insignificantly increased STS among workers exposed to multiple phenoxy herbicides and chlorophenols (Saracci *et al*., 1991), and slightly significantly increased NHL associated with number of acres sprayed with herbicides (Wigle *et al*., 1990).

The NCI Studies

In the early 1980s, the NCI of the U.S. embarked on a relatively large program aimed at testing the hypotheses raised by Hardell's work regarding phenoxy acid herbicides and STS, HD and NHL through a series of case-control studies of farmers in Kansas, Nebraska, Iowa, and Minnesota, and with exposure information gathered through questionnaires.

In 1986, NCI investigators published the results of the case-control study of Kansas farmers (Hoar *et al.*, 1986). The investigators were unable to find evidence of an association between phenoxy herbicides and either HD or STS. They did report, however, a suggestive association between reported usage of herbicides in farming and NHL (OR[7] 1.6, CI 0.9-2.6). The authors reported that the relative risk of NHL increased significantly with number of days of herbicide exposure per year and latency. Men exposed to herbicides more than 20 days per year had a six-fold increased risk of NHL (OR 6.0, CI 1.9-19.5) relative to non-farmers. Frequent users who mixed or applied the herbicides themselves had an OR of 8.0 (CI, 2.3-27.9) for NHL. The authors interpreted the data to suggest that the excesses were associated with use of phenoxy herbicides, especially 2,4-D, although no questions pertaining specifically to frequency of use of 2,4-D were part of the interview instrument used to assess potential exposure.

The Kansas study was a case-control effort with cases and controls being selected from population-based registries. Rigorous field interviews were conducted, and appropriate quality control procedures were employed. The study presents a difficult interpretational problem, however, because exposure information was gleaned exclusively from interviews of subjects or their next of kin.

The finding of increased risk of NHL in the Kansas study was based on seven cases reporting usage for more than 20 days per year, with information on this usage for three of those cases coming from next-

[7]odds ratio

of-kin respondents. Other surrogates in the study for intensity of exposure, such as number of years spraying, did not suggest a dose-related risk. Subsequent independent reviews of this study by the EPA, the Ontario Ministry of Environment, Agriculture Canada and the Council for Agricultural Science and Technology were conducted (Burmeister, 1986; MacMahon, 1986; 1990; Morgan, 1986; Linet, 1986; CCT, 1987; Council for Agricultural Science and Technology, 1987; EPA, 1988; Agriculture Canada, 1989; Ibrahim *et al.*, 1991). During the course of these reviews, it became clear that the reported exposure scenario of 21 or more days of use per year on a farm was probably unrealistic for the majority of farmers, since the need for the type of weed control offered by the phenoxy acids covers a narrow time window.

In addition, one of the most interesting findings with respect to the cause-effect hypothesis is the observed dose-response. Risks of NHL increased from 1.4 to 6.0 along four categories of exposure based on reported days per year of usage. The odds ratio in the highest exposure group reached statistical significance. However, it should be noted that misclassification along this reported exposure gradient of but one or two cases would result in the finding of increased risk either disappearing or losing its statistical significance. In addition, the interview question relating to frequency of use was not specific in that it queried regarding frequency of use of herbicides in general. The questions did not seek frequency-of-use information specific to 2,4-D.

The results of any case-control study, such as the NCI studies, relying on the ability of subjects to recall past exposures must be interpreted carefully. The problem grows worse when proxy respondents are asked to recall the past exposures of the subjects.

For example, in the Kansas study, farmers and the suppliers of insecticides and herbicides could only agree on the farmers' usage 50 to 60% of the time (Blair and Zahm, 1992), suggesting that subjects might not be remembering their past exposures accurately. In data from Iowa and Minnesota, proxies were twice as likely as subjects not to know if a specific pesticide had been used (Blair and Zahm, 1992). During the last year, researchers from the U.S. Centers for Disease Control also reported that proxies often were unaware of pesticide use (Boyle *et al.*, 1991).

The issue has not been settled, however. Also during the last year, researchers from the NCI reported that agreement between subjects and proxies was excellent for yes/no questions regarding specific

pesticides, although agreement was much less for number of days of use (Brown *et al.*, 1991).

If there were no differences in recollection between cases and controls, inaccurate recall of exposure would be nondifferential misclassification that would reduce risk estimates, and this explanation has been advanced (Blair and Zahm, 1992).

Other epidemiologists and data suggest, however, that cases and controls, or their proxies, remember things differently; this differential misclassification could have resulted in overestimated risks (Johnson *et al.*, in press; Olsen, 1992). Johnson *et al.* (in press) also report that when nondifferential misclassification occurs, the risk estimate may not always be biased toward the null value.

After considering these and other interpretational issues, the scientific panel reviews of the Kansas farm worker study each concluded that the study was not persuasive in suggesting a link between 2,4-D and cancer.

The NCI investigators published data pertaining to Nebraska farmers in 1990, another case-control study (Zahm *et al.*, 1990). The Nebraska farmworker study sought additional information on frequency of use of specific herbicides such as 2,4-D. The investigators gathered data on HD and NHL, but not on STS; only the NHL data were reported. The investigators reported again, as they had for their Kansas study, that they had observed a trend suggesting increasing NHL risk with increasing days per year of reported use of 2,4-D. The trend was statistically significant; however, it was driven by only three cases of NHL reporting usage of 2,4-D of 21 or more days per year. Data from proxy respondents gave a higher estimated risk than data from subjects. A review of this study by Dr. Brian MacMahon of the Harvard School of Public Health revealed that the suggestion of increased risk was almost entirely the result of next-of-kin responses. No trend of increasing risk with increasing days of use was evident when cancer cases themselves reported historical exposures (MacMahon, 1990). The apparent consistency among the Kansas and Nebraska studies would have been more notable if the two studies had not been conducted by the same investigators.

During the fall of 1989, the Harvard School of Public Health convened a panel of scientific experts, chaired by Professor Michel Ibrahim of the University of North Carolina School of Public Health, to review both the Kansas and Nebraska case-control data in the context of the weight of scientific

evidence regarding 2,4-D. The panel concluded that the link between 2,4-D and cancer was far from established (Ibrahim *et al.*, 1991).

In 1991, Hayes *et al.* of the NCI published a case-control study of malignant lymphoma among dogs that was offered as further evidence of a link between 2,4-D and human lymphoma. The investigators studied companion dogs to examine the risk of developing canine malignant lymphoma associated with the use of chemicals in and about the home. Information from a general self-administered owner questionnaire and/or a telephone interview of about 491 cases, 466 non-tumor controls, and 479 tumor controls indicated that owners in households with dogs that developed malignant lymphoma applied 2,4-D-containing herbicides to their lawn and/or employed commercial lawn care companies to treat their yard significantly more frequently than control owners (OR 1.3). In addition, the authors reported that the risk of canine malignant lymphoma rose to a two-fold excess with four or more yearly owner applications of 2,4-D. The authors maintained that their data indicated that human health implications of 2,4-D exposure in the home environment should receive further investigation.

The Industry Task Force II on 2,4-D Research Data commissioned an expert panel review of the dog study during 1991 (Carlo *et al.*, in press). Among a number of issues inherent to the study design and implementation, documentation and quantification of exposure to 2,4-D was most problematic. The questionnaire employed to elicit information on exposure was non-specific with respect to herbicide exposure, and it is unclear whether the exposure variable employed in the study reflected to any degree true underlying exposures to 2,4-D. The expert panel concluded that due to serious limitations in the design of the study, the data did not show an association between dog owners' use of 2,4-D and canine malignant lymphoma.

In 1992, investigators from the NCI published their findings with respect to Iowa and Minnesota farmers, and reported only limited information relevant to the 2,4-D and cancer hypothesis (Cantor *et al.*, 1992). Data were gathered from interviews with 622 white men with newly diagnosed NHL and 1245 population-based controls. Men who had ever farmed had a slightly, but significantly, elevated risk of NHL (OR 1.2, CI 1.0-1.5). No significant risk increase associated with use of 2,4-D was identified (OR 1.2, CI 0.9-1.6), and this did not change with latency or use of protective equipment. Specific information on the frequency of use of 2,4-D and other herbicides and risk of NHL was not reported. The investigators stated that they adjusted for proxy respondents in their analysis, but no

details were given.

In their study on methodology, Johnson *et al.* (in press) compared the responses given during 1981-1983 by the Minnesota participants to the responses given during 1990-1991 by proxies for those participants. The data indicated that both nondifferential and differential misclassification occurred when proxies provided pesticide exposure data.

Taken as a group, the series of case-control studies published by investigators from the NCI were informative with respect to risks of NHL and farming. Because exposure information on past herbicide use gathered through questionnaire and/or next-of-kin recall can be inaccurate, the studies add limited information with respect to the specific hypothesis that 2,4-D is a cause of human cancer.

The Agent Orange Studies

The international interest in the potential effects of exposure to the herbicide Agent Orange used as a defoliant during the Vietnam war, and subsequent epidemiology studies aimed at assessing health risks of this exposure, have provided a database useful relative to the 2,4-D cancer hypothesis.

Termed Agent Orange because of the orange stripe painted on barrels used to transport it, the 50-50 mixture of the phenoxy herbicides 2,4-D and 2,4,5-T was used widely during the Vietnam war to defoliate jungles as a means of avoiding ambush. Studies mandated by the U.S. and Australian governments, as well as other state authorities, have provided much useful information directly relevant to 2,4-D. While studies of ground troops have proven difficult from the perspective of documenting exposure, studies of military personnel involved in day-to-day spraying of the herbicides provide the most rigorous scientific data available.

The Project Ranch Hand studies being conducted by the U.S. Air Force address health effects among a cohort of soldiers likely to have been exposed to considerable amounts of phenoxy herbicides through aerial spraying. Wartime and climatic conditions caused this group to be exposed to large amounts of phenoxy herbicides. Note, however, that these studies are of small size and have limited statistical power to detect small increases in risk.

The first report on Project Ranch Hand veterans was released in 1983 (USAF School of Aerospace Medicine, 1983). The analysis of deaths among 1240 veterans showed that the mortality experience of the Ranch Hand group was nearly identical to that of the comparison group, Air Force personnel not involved in Project Ranch Hand. The Ranch Handers showed a relative paucity of overall cancer but an excess of digestive disorder deaths, neither statistically significant. No STS or lymphatic cancer deaths were detected in either group. Because the cohort was young at the time of the first study, ongoing surveillance and follow-up have continued.

In 1985, Wolfe and Michalek reported further on the 20-year comprehensive assessment of the health of Air Force veterans of Operation Ranch Hand. The follow-up study compared the non-combat mortality of Ranch Hand veterans with a comparison group of Air Force veterans primarily involved with cargo missions in Southeast Asia, but who were not exposed to herbicides. There were no statistically significant differences in mortality between the Ranch Hands and the comparison group. No STS or lymphatic cancer deaths were detected in the Ranch Hands, while one STS and five lymphatic/hematopoietic cancers were detected in the comparison group.

Further health and mortality follow-ups of the Ranch Hand cohort were published in 1990 by Wolfe *et al*. and Michalek *et al*., respectively. Nine hundred ninety-five Ranch Hands and 1299 comparison subjects attended the follow-up examination. The two groups were similar in reported health problems, diagnosed skin conditions, and hepatic, cardiovascular, and immune profiles. Ranch Hands experienced significantly more basal cell carcinomas than comparison subjects. The two groups were not different with respect to melanoma and systemic cancer. One NHL and one soft tissue cancer, but no HD were seen in the Ranch Hands; no NHL but one soft tissue cancer and one HD were seen in the comparison group.

The mortality report (Michalek *et al.*, 1990) compared the non-combat mortality of 1261 Ranch Hand veterans to that of a comparison population of 19,101 other Air Force veterans primarily involved in cargo missions in Southeast Asia, but who were not exposed to herbicides. The indirectly standardized all-cause death rate among Ranch Hands was reported as 2.5 deaths per 1000 person-years; comparison subjects had a similar all-cause death rate. After adjustment for age, rank, and occupation, the all-cause SMR was 1.0. In adjusted cause-specific analyses, the authors reported no significant group differences regarding accidental, malignant neoplasm or circulatory deaths. One Ranch Hand

and one comparison subject had died of STS. No Ranch Hands had died of lymphatic cancer, while 10 comparison subjects had. The authors concluded that these data are not supportive of a hypothesis of increased mortality among Ranch Hand veterans exposed to herbicides.

Overall, the Ranch Hand studies of individuals with known and considerable exposures have failed to show an association between phenoxy acid herbicides and any reported health effects. Because the population being studied is relatively small, however, these studies truly provide information only on the absence of large risks.

Studies of the Vietnam Army Chemical Corps veterans provide information on foot soldiers. Thomas and Kang (1990) reported on the mortality and morbidity among Army Chemical Corps, nearly 1,000 men serving between 1965 and 1971 who were responsible for the mixing and application of herbicides, riot control substances and burning agents. Information on Vietnam service was obtained from military records of 94% of this cohort. Follow-up for vital status on December 31, 1987 was conducted using Department of Veterans Affairs, military, National Death Index, U.S. Internal Revenue Service and Social Security Administration records. Cause-specific observed numbers of deaths among the 894 men included in the study group were compared with the numbers expected based on rates for U.S. men, adjusting for race, age, and calendar period. Fifty-three deaths from all causes were observed during the study period, compared to 48.8 expected (SMR 1.09). There were statistically significant excesses of digestive disease deaths (SMR 2.98), primarily due to cirrhosis, and from motor vehicle accidents (SMR 2.00). Two deaths were observed from leukemia (0.5 expected) and two from brain cancer (0.4 expected). Two HD were observed in living veterans, where less than one was expected. The authors concluded that because of the small study group size and the lack of specificity of information regarding their exposures, their findings cannot be attributed to any single chemical agent.

Studies of other ground troops provide information relevant to the Vietnam experience; however, those studies provide little useful information regarding the specific 2,4-D cancer hypotheses (see Table 2).

Expert panel reviews of the epidemiology relating 2,4-D to cancer

Overall, a considerable number of human epidemiology studies relevant to assessing the 2,4-D cancer hypothesis have been conducted throughout the world. Several expert scientific panels have reviewed the weight of this evidence, and none have concluded that 2,4-D is a cause of human cancer (Burmeister, 1986; MacMahon, 1986; 1990; Morgan, 1986; Linet, 1986; CCT, 1987; Council for Agricultural Science and Technology, 1987; EPA, 1988; Agriculture Canada, 1989; Ibrahim *et al.*, 1991).

The epidemiology studies support the concept that broadly defined occupational groups such as farmers and forestry workers may be at increased risk for certain types of cancers, particularly lymphopoietic cancer. However, more refined studies capable of addressing the 2,4-D cancer hypothesis with the benefit of more reliable exposure data yielded mostly negative, but some inconsistent, findings on the relationship between 2,4-D and the risk of cancer.

More information can be expected from the five-year prospective cohort study of 100,000 farmers and their families that the NCI, the National Institute of Environmental Health Sciences and the EPA are beginning (Science, 1992). It is unclear if even this study will be conclusive with respect to 2,4-D, however, since it will examine a number of cancers (including leukemia, melanoma, brain cancer, HD and multiple myeloma) and a number of exposures (including pesticides, fertilizers, ultraviolet light, dust and viruses).

From the perspective of public health protection, even if the hypothesis that 2,4-D use for 21 or more days per year in farming were assumed to be valid, the exceedingly small number of persons likely to engage in such a practice leads to a population attributable risk that is diminishingly small. With the new label requirements and other exposure reduction measures that have been introduced, few, if any, cases of NHL could be attributed to continued use of 2,4-D.

Animal bioassays on 2,4-D

Long-term bioassays have been carried out using rats, mice and dogs, and do not suggest that 2,4-D is an animal carcinogen. No evidence of neoplastic or preneoplastic effects on "classical" target organs have been observed in any species. The only finding of concern was an apparent increase in the incidence of brain astrocytomas in rats treated at the highest 2,4-D dose of 45 mg/kg body weight/day observed in one study (ITF, 1986); however, the biological characteristics of the tumors were not consistent with chemical carcinogenesis. Based on the lack of decreased latency, the lack of increased multiplicity, the lack of increased severity, the lack of preneoplastic or target organ effects, the restriction of tumor development to one sex, the intergroup variability exhibited among historical controls, the lack of a plausible mechanism of tumorigenesis and the low exposure of the brain to 2,4-D compared to other tissues, it appears very unlikely that the increased incidence of brain astrocytomas observed in this study is related to 2,4-D treatment. In addition, 2,4-D is not structurally related to known brain carcinogens, all of which appear to act through the formation of an electrophilic intermediate and affect other sites in addition to the brain. This provides further support for the contention that the astrocytomas in 2,4-D-treated rats were incidental. Overall the results from animal bioassays with 2,4-D are consistent with the position that 2,4-D is not an animal carcinogen, and provide no reason to suspect that 2,4-D might be a human carcinogen.

Specific Studies

Three long-term bioassays have been carried out in the rat (Hansen *et al.*, 1971; Archipov and Kozlova, 1974; ITF, 1986). In the first of these, groups of 25 male and 25 female Osborne-Mendel rats were administered 2,4-D via the diet for two years at concentrations of 0, 5, 125, 625 or 1250 ppm (approximately 0.25, 6.25, 31.2 and 62.5 mg/kg body weight/day) (Hansen *et al.*, 1971). All animals were killed and necropsied after two years, except for one high dose rat which died during the experiment. Microscopic examinations were conducted on all tumors, heart, lung, liver, spleen, kidney, stomach, intestines, pancreas, pituitary, thyroid, adrenal, bone, ovary, and uterus (or testis and prostate) as well as tissues with gross lesions from six animals of each sex of the high dose and control groups. Tumors, liver, kidney, spleen, ovary (or testis), and tissues with gross lesions from animals in other dose groups were subjected to microscopic examination. Treatment with 2,4-D did not affect survival, body weights or organ weights in any dose group. The total number of male rats with

malignant tumors was increased in the high dose group and a trend toward increased tumor development was associated with log dose in females; however, the tumors were randomly distributed among tissues normally developing tumors in aging rats and there was no evidence of a target organ effect (Hansen *et al.*, 1971). The results of this study provide no evidence that 2,4-D is carcinogenic in the rat, although this cannot be considered a definitive study since it was not conducted under the requirements of Good Laboratory Practice (GLP) standards, the dose groups were fairly small, the maximum tolerable dose was not achieved and microscopic evaluations were not made of a comprehensive list of tissues and organs.

In a further account of this same study by Reuber (1983), the author draws attention to an apparent increase in the incidence of lymphosarcomas which was not considered significant in the original study report authored by Hansen *et al.* (1971). The incidences of lymphosarcomas in male and female rats were as follows: in males, 0, 8, 16, 20, 13 and 26%, and in females, 0, 25, 27, 26, 50 and 24% at 0, 5, 25, 125, 625 and 1250 ppm in the diet respectively. In males the tumor incidence was significant at $p<0.05$ at the 125 and 1250 ppm dietary levels, but not at 5, 25 or 625 ppm, while in females significance was reached at all dose levels. The tumor incidence in males increased slightly between the dietary levels of 5, 25, and 125 ppm, decreased at 625 ppm and increased again at 1250 ppm. There was only a three-fold increase in lymphosarcoma incidence between the 5 and 1250 ppm levels, even though this represented a 250-fold increase in exposure. The highest dietary level of 1250 ppm would result in a dose of approximately 63 mg/kg body weight. This exceeds the threshold of 50 mg/kg body weight for renal tubular secretion in the rat, and therefore would be expected to result in a non-linear increase in systemic exposure to 2,4-D and a disproportionate increase in treatment-related tumor incidence. Such a disproportionate increase in lymphosarcoma incidence did not occur in males at the highest dose. In fact, the increase relative to the incidence at a 10-fold lower dose was very slight. In females the tumor incidence actually decreased at 1250 ppm compared to 625 ppm. This occurred even in the absence of any noticeable toxicity. It is evident from this evaluation that there is no clear relationship between exposure of the rats to 2,4-D and development of lymphosarcomas. In order to properly assess the significance of this apparent increase in tumors in treated animals, a comparison with historical control data must be made (Burek *et al.*, 1988; van Zwieten *et al.*, 1988). Historical control data for Osborne-Mendel rats may have been available to the original study author, since mention is made to normal sites of tumor development in aging rats (Hansen *et al.*, 1971), although the data are not presented. No consideration was given to historical control data by Reuber (1983) in his

evaluation. Tumors of the hematopoietic system, including lymphoma and leukemia are very common in aging F344 rats (Goodman *et al.*, 1979). The incidence of lymphoma and leukemia in control male rats used in the NCI/NTP (National Toxicology Program) bioassay program was 23.3% among 4004 rats (Haseman, 1983). Based on this it seems likely that the incidence of 0% in controls in the study using Osborne-Mendel rats is below the expected rate, although data for control Osborne-Mendel rats were not available for confirmation of this.

The absence of a clear dose response over a wide range of exposures in both male and female rats, and the likelihood that the control incidence was unusually low, strongly indicate that the lymphosarcomas observed in these rats were not related to 2,4-D treatment, in agreement with the original interpretation of the study results by Hansen *et al.* (1971). It should also be noted that the review by Reuber (1983) is not published in a toxicology journal, and does not provide balanced, comprehensive coverage of the 2,4-D database. Some additional doubt regarding the significance of the lymphosarcomas is raised due to the fact that no increase in lymphosarcomas was observed in a comprehensive GLP study using another strain of rat as discussed below (ITF, 1986).

The second 2,4-D feeding study was poorly reported and not carried out according to GLP requirements. A group of 120 male and 45 female rats were administered an oral dose of 10% of the LD_{50} for life (dosing details not specified), with no significant increase in tumor incidence (Archipov and Kozlova, 1974).

A third rat study, conducted by Hazleton Laboratories under contract to the Industry Task Force on 2,4-D Research Data, was conducted according to GLP requirements (ITF, 1986). Groups of 60 male and 60 female F344 rats were administered 2,4-D via the diet at dose levels of 0, 1, 5, 15, or 45 mg/kg body weight/day for two years. Hematology, clinical chemistry and urinalysis parameters were evaluated prior to dosing and after 26, 52 and 78 weeks of treatment. Necropsies were conducted on all animals that died or were killed *in extremis*, 10 animals per sex per group after 52 weeks and all surviving animals after two years. Microscopic examinations were performed on a comprehensive list of tissues and organs. There were no clinical changes, effects on hematology, clinical chemistry or urinalysis parameters, survival or gross findings associated with 52- or 104- week exposure to 2,4-D at any dose level. Body weight gains were significantly reduced relative to controls in high dose females at both time points. Food consumption was also significantly reduced in high dose females during the

first 52 weeks of treatment. Body weights and food consumption were not significantly affected in high dose males or in lower dose groups of either sex. With the exception of the kidney, there were no biologically significant effects on organ weights. Absolute and relative kidney weights were increased in males of the 15 and 45 mg/kg body weight/day dose groups after 52 or 104 weeks of treatment, and in all groups of treated females after 104 weeks. Histopathological changes were also observed in the kidneys in all except the low dose groups of both sexes. These changes included increased incidence of brown tubular pigment and increased severity of fine cytoplasmic vacuolization in the renal cortex, and were observed after 52 or 104 weeks of treatment. After 104 weeks there was an increase in frequency of pelvic microcalculi in males of the 15 and 45 mg/kg body weight/day dose groups and in females of the 45 mg/kg body weight/day dose group. High dose females also exhibited an increased incidence of renal pelvic transitional cell hyperplasia, considered to be secondary to the presence of microcalculi.

Tumor incidences were not reported to be increased relative to controls with the exception of brain astrocytomas in male rats. The incidences of these tumors were 1/60, 0/60, 0/60, 2/58, and 6/60 in the control, 1, 5, 15, and 45 mg/kg body weight/day groups, respectively. These data showed a significant positive trend and the increase in the high dose group was reported to be statistically significant ($p=0.05$). While the statistical analysis of the tumor data may suggest a treatment-related effect, the biological characteristics do not. Assessment of biological evidence should take precedence over statistical analysis in attempting to distinguish between treatment-related and spontaneous brain neoplasms since there is a high degree of variability in brain tumor incidence among rats, and a greater chance of random occurrence of increased tumor incidence among one of several treated groups than in a single control group in a given study (Koestner, 1986). The tumors in treated animals did not occur earlier than in controls, did not display multiplicity, were not larger or more anaplastic than in controls and were significantly increased only in one sex. The largest and most severe tumor was actually found in a control animal. In addition, there was no evidence of lesion development in any of the animals without tumors, or brain cell toxicity.

The brain receives a lower dose of 2,4-D than other organs in which tumors did not develop, 2,4-D is not metabolized to an active intermediate, is rapidly eliminated, does not accumulate in body tissues, and gives generally negative results in genotoxicity assays. The lack of target organ toxicity, the low target organ exposure, the rapid excretion, the absence of a potential reactive intermediate and the lack of genotoxicity preclude the hypothesis of any plausible mechanism of tumorigenesis of 2,4-D. This

indicates that 2,4-D is quite different from known brain carcinogens, all of which appear to act through the formation of an electrophilic intermediate (Kleihues *et al.*, 1982; Swenberg, 1986), and affect other sites as well as the brain (Kleihues *et al.*, 1982; Swenberg, 1986; Ward and Rice, 1982). The incidence of astrocytomas in the 2,4-D study was 3.6% based on six to eight brain sections per rat and 2.8% based on four brain sections per rat. This is higher than the overall historical control incidence for F344 rats in the NTP database of 0.8%, based on four brain sections per animal (Solleveld *et al.*, 1984); however, it is similar to the brain tumor incidence of 3.3% among 180 control male F344 rats reported by the NCI/NTP contract laboratory observing the highest incidence of these tumors (Ward and Rice, 1982). Significant intergroup variability has been reported for brain astrocytomas in Fisher rats (Solleveld *et al.*, 1984), necessitating the consideration that statistically significant false positive findings are possible for this type of tumor. The lack of a plausible mechanism of tumorigenesis along with the fact that the biological characteristics of the astrocytomas are not characteristic of chemically induced tumors suggests that the brain tumors observed in 2,4-D-treated rats are unlikely to have been caused by exposure to 2,4-D. This conclusion was also arrived at by A. Koestner in an independent expert review of the astrocytoma data from the ITC (1986) study (Koestner, unpublished).

Two long-term bioassays have been carried out using mice. In the first, groups of 18 mice of each sex from two different strains ((C57BL/6 x C3H/Anf)F_1 and C57BL/6 x AKR)F_1) were given oral doses of 2,4-D isopropyl ester, butyl ester or isooctyl ester at the previously determined maximum tolerable dose of 46.4 mg/kg body weight/day for 18 months (Innes *et al.*, 1969). After sacrifice, the thoracic and abdominal cavities were examined and microscopic examinations were carried out on major organs and gross lesions. Tumor yields were analyzed under the categories hepatomas, pulmonary tumors, lymphomas and total. No significant increase in tumor incidence was observed in either strain of mice, with any of the 2,4-D compounds tested.

The second mouse study was carried out according to GLP requirements in conjunction with the GLP rat study discussed above (ITF, 1986). Groups of 60 B6C3F1 mice of each sex were given 2,4-D via the diet at doses of 0, 1, 15, or 45 mg/kg body weight/day for 106 weeks (ITF, 1987). Parameters evaluated to assess toxicity and carcinogenicity were the same as in the companion rat bioassay discussed previously (ITF, 1986). There were no treatment-related effects on survival, body weights, clinical chemistry, hematology, urinalysis parameters, or gross pathology. Microscopic evaluation revealed an increase in cytoplasmic homogeneity in the renal tubular epithelium in male mice of the top

two dose groups. This finding appears to be treatment related, but the significance is unclear. There were no increases in tumor incidences in any group.

The effects of long-term exposure to 2,4-D has also been studied in dogs. Groups of three Beagle dogs of each sex were administered 2,4-D in the feed at concentrations of 0, 10, 50, 100, or 500 ppm for two years (Hansen *et al.*, 1971). Gross and microscopic examinations were performed on the following tissues and organs: heart, lung, liver, spleen, gall bladder, kidney, pancreas, adrenal, thyroid, pituitary, cervical lymph node, mesenteric lymph node, submaxillary lymph node, intestine, stomach, bone, brain, spinal cord, bladder, salivary gland, testis and prostate, or ovary and uterus. No lesions associated with 2,4-D administration were observed. While there were no tumors associated with 2,4-D treatment, this cannot be considered a definitive study since so few animals were used.

EVALUATION OF NON-CANCER ENDPOINTS

Other health effects, where there was an adequate body of evidence to base an evaluation, are discussed in the following sections. These endpoints included subchronic and chronic toxicity, neurotoxicity, immunotoxicity, and reproductive toxicity.

Subchronic and chronic studies

The two main target organs for 2,4-D toxicity are the kidney and the thyroid, with the kidney being the most sensitive. The weight-of-evidence from a number of studies supports the conclusion that the subchronic no-observed-effect level (NOEL) in rats and mice is 15 mg/kg body weight/day. At higher doses causing saturation of renal tubular secretion, there is a loss of epithelial cells in the brush border of the proximal tubule in the kidney of both species, and an increase in the incidence of thyroid follicular cell hypertrophy in the rat. In the dog, a NOEL of 1 mg/kg body weight/day has been established on the basis of a single 13-week study employing five animals per sex. Subtle histologic changes in the kidney of male and female dogs at a dose of 10 mg/kg body weight/day and in males at a dose of 3 mg/kg body weight/day were characterized by alteration in the staining properties of epithelial cells lining some convoluted tubules.

Evaluation of Subchronic Dose-Response

Subchronic animal bioassays have been carried out with 2,4-D, its salts and esters, using various animal species exposed through various routes. The protocols of these studies are summarized in Table 4.

The results of a number of three-week dermal application studies using rabbits indicate that systemic effects, including overt signs of toxicity as well as effects on the kidney, are observed at doses of 1000 mg/kg body weight/day or more (ITF, 1992). At lower doses, only local effects on the skin were observed. NOELs of 18 and 16.3 mg/kg body weight/day were reported for the dimethylamine salt and the ethylhexyl ester respectively in rabbits dermally exposed for three weeks (ITF, 1992). The results of these studies indicate that systemic effects from dermal exposure occur at much higher dose levels than do local dermal effects, and that thresholds exist below which dermal exposure causes no effects.

The data most suited to evaluation of the 2,4-D dose-response come from a group of 13-week oral studies, since unlike the situation with the dermal studies, the actual systemic dose is known.

Subchronic toxicity studies in which groups of 10 male and 10 female F344 rats were treated with 2,4-D via the diet have identified the NOEL as 15 mg 2,4-D/kg body weight/day for the acid (ITF, 1991a), the ethylhexyl ester (ITF, 1991b), the diethylamine salt (ITF, 1991c), the butoxyethyl ester (Dow, 1991a), the triisopropanolamine salt (Dow, 1991b), and the isopropylamine salt (Dow, 1991c). At higher doses, in excess of the threshold for saturation of renal clearance, the two key target organs appear to be the kidney and the thyroid, with the kidney being the most sensitive. Effects on other organs at higher doses were secondary to overt toxicity. No difference in toxicity would be expected among the different forms of 2,4-D based on evidence from metabolism and pharmacokinetics studies, in which it has been shown that the excretion kinetics are similar for all forms and that only 2,4-D acid is detectable in blood or urine soon after dosing with other forms.

In the kidney there was loss of epithelial cells in the proximal tubule brush border, and in the thyroid there was follicular cell hypertrophy in association with a reduction in serum thyroxine levels. These findings were consistent across all the forms of 2,4-D tested and occurred at dose levels in excess of the threshold for saturation of renal tubular secretion (Dow, 1991a,b,c; ITF, 1991a,b,c). In earlier studies with the acid that employed similar protocols, effects on the kidney and thyroid were reported

at lower doses than in the more recent studies (Gorzinski *et al.*, 1981a,b; ITF, 1983a). Detailed summaries of the effects reported in the kidneys and thyroid in these studies are shown in Tables 5 and 6, respectively. Kidney effects reported by Gorzinski *et al.* (1981a,b) included increases in organ weights and increased incidences of histological changes described as epithelial cytoplasmic homogeneity of convoluted tubules in males, and epithelial cytoplasmic vacuolization of convoluted tubules in females. These changes were not severe, being described as multifocal and slight even at the highest doses (see Table 5). The incidences of these histological changes were not significantly increased over controls in males or females treated with purified (Gorzinski *et al.*, 1981a) or technical grade (Gorzinski *et al.*, 1981b) 2,4-D at a dose of 15 mg/kg body weight/day. This is consistent with the NOEL of 15 mg/kg body weight/day observed in the recent studies (Dow, 1991a,b,c; ITF, 1991a,b,c). Increases in relative, but not absolute kidney weights were observed in males at the 15 mg/kg body weight/day dose level. This finding is inconsistent with the lack of change in kidney weights in females at this dose level (Gorzinski *et al.*, 1981a,b), and the similar lack of kidney weight change at the 15 mg/kg body weight/day dose level in seven more recent studies (Dow, 1991a,b,c; ITF, 1983a; ITF, 1991a,b,c). In one study, minor histological changes were reported in the kidneys of male rats at the 5 mg/kg body weight/day dose level and in one female at the 1 mg/kg body weight/day dose level (ITF, 1983a). These changes were different from those reported in any of the other eight similarly conducted studies, and were described as increased homogeneity, altered tinctorial properties and fine vacuolization of the cytoplasm in the cortex. In all the other studies, histological changes were increased relative to controls only at doses in excess of the threshold for saturation of renal tubular secretion, and changes affected the tubules, rather than the glomeruli (Dow, 1991a,b,c; Gorzinski *et al.*, 1981a,b; ITF, 1991a,b,c). 2,4-D is excreted through renal tubular secretion, therefore, it stands to reason that overloading the secretion mechanism may cause damage to the renal tubules. A physiological basis for cortical changes, which may be suggested by the results of the ITF (1983a) study, is not readily postulated. Although the discrepancies among the three older studies (Gorzinski *et al.*, 1981a,b; ITF, 1983a) and the six recent studies (Dow, 1991a,b,c; ITF, 1991a,b,c), are not easily explained, the weight-of-evidence supports the conclusion that the NOEL for subchronic 2,4-D exposure in the rat is 15 mg/kg body weight/day.

Thyroid changes have been reported in one rat study at doses of 15 mg/kg body weight/day or lower (ITF, 1983a). Effects on the thyroid reported in this study are summarized in Table 6. The highest dose used in this study was 45 mg/kg body weight/day, and resulted in increased organ weight in males, and no effects on the thyroid in females. The reported increase in organ weights did not show a clear dose response and was not associated with histological changes. Serum T4 levels were increased in males of the 5 and 15 mg/kg body weight/day dose levels, but the changes were not dose-related and were inconsistent with the decreases in thyroxine levels reported at higher doses in other studies (Dow, 1991a,b,c; Gorzinski *et al.*, 1981a,b; ITF, 1991a,b,c). The increases in serum T4 in males and the increases in thyroid weight in males and females reported in the ITF (1983a) study do not clearly suggest a relationship with treatment and are inconsistent with the results of eight other studies, therefore, it appears that the changes noted in this study are incidental and do not affect the conclusion that the subchronic NOEL for 2,4-D in the rat is 15 mg/kg body weight/day.

In mice, two 13-week studies have been carried out at doses below those associated with signs of overt toxicity. Both used B6C3F1 mice administered 2,4-D acid in the diet. In the first of these, histopathological changes in the kidney, similar to those observed in rats, were observed at doses of 5 to 90 mg/kg body weight/day (ITF, 1983b). In a more recent study using dose levels of 1, 15, 100 and 300 mg/kg body weight/day, a NOEL of 15 mg/kg body weight/day was determined, with kidney effects observed only at the highest dose level. This is the consistent with the NOEL of 15 mg/kg body weight/day established in the rat. The reason for the apparent variability in toxicity between the two studies is unclear.

One subchronic study has been conducted in dogs, in which groups of five Beagles per sex received oral doses of 0, 0.3, 1, 3 or 10 mg/kg body weight/day 2,4-D acid in gelatin capsules (ITF, 1990). Subtle histologic kidney changes were observed in males of the two highest dose groups and in females of the highest dose group. These changes involved reduced cytoplasmic eosinophilia of the epithelial cells lining some convoluted tubules. The pathogenesis of the lesions could not be determined, but the changes were considered to be similar to those seen in regenerative tubular epithelium (ITF, 1990). In the 10 mg/kg body weight/day dose group three
out of three males and one out of four females were affected, and in the 3 mg/kg body weight/day dose group three out of five males were affected. The NOEL in this study was 1 mg/kg body weight/day.

Neurotoxicity studies

In the scientific literature there are several reports of a possible association between exposure to 2,4-D in humans and the development of neuropathological and neurobehavioral abnormalities. Some of the alleged effects of 2,4-D exposure in humans include: the development of persistent peripheral polyneuropathy (Young *et al.*, 1978; AMA, 1981; Reuber, 1983; Fleck, 1985), demyelination and ganglion degeneration in the central nervous system (Lathrop *et al.*, 1982, Reuber, 1983; Green, 1987), reduced nerve conduction velocity (Singer *et al.*, 1982), myotonia[8] (Buslovich and Pichugin, 1983), and suicide in forestry workers (Green, 1987; 1991) and depression, suicide, anxiety, aggression and other features associated with post-traumatic stress syndrome in Vietnam veterans (Clement Associates, 1985; 1989; 1990; Levy, 1988).

Of the alleged effects, only myotonia has been verified in experimental animal bioassays (Steiss *et al.*, 1987; Arnold *et al.*, 1991; Beasley *et al.*, 1991). The other reported effects of 2,4-D exposure in humans were either from case-reports (for demyelination, ganglion degeneration, and peripheral polyneuropathy) which provided few details of a cause-and-effect relationship or were from epidemiological studies (involving slowed nerve conduction velocity, suicide, and post-traumatic stress disorder) in which:

1) the exposed population may not have been representative of the general population (*e.g.*, Vietnam veterans *versus* non-Vietnam veterans),
2) there were potentially confounding exposures to other chemicals (2,4,5-T and PCDD),
3) the toxicological effects were attributed to 2,3,7,8-T_4CDD rather than 2,4-D, and/or
4) there was inadequate quantification of the 2,4-D exposures involved.

As a result of these deficiencies, and given the lack of supporting evidence in experimental animals, these epidemiology studies offer little information regarding 2,4-D and neurological effects.

Overall it may be concluded that 2,4-D has little, if any, potential to induce neurotoxicity at doses which do not cause overt systemic toxicity or saturation of processes involved with tissue clearance and excretion. At doses above 100 mg/kg body weight, 2,4-D can accumulate in brain tissue as a

[8]it was unclear from this abstract of the Russian study whether the effects were observed in humans or animals

result of the inhibition of the anionic transport processes responsible for clearing acidic metabolites from the brain. Also, at doses in excess of 100 mg/kg body weight, 2,4-D can induce myotonia of skeletal muscle. At doses at or below the range of doses associated with normal renal clearance (*e.g.*, 30 to 50 mg/kg body weight), there is no evidence in experimental animals to indicate that 2,4-D can induce neurotoxicity. In humans, the few available case reports do not provide sufficient evidence for the establishment of a cause-and-effect relationship between 2,4-D exposure and the development of peripheral polyneuropathy.

Specific Studies

Five reports of epidemiological studies have addressed the possibility that 2,4-D is a neurotoxicant. (See Table 7). The epidemiological data, however, are inadequate to draw conclusions regarding these outcomes.

Case reports have suggested an association between accidental acute 2,4-D exposure and the development of peripheral neuropathy. This potential effect of 2,4-D has not been substantiated in a number of animal studies using doses many times those to which humans were exposed. In addition, the very low frequency of reports of polyneuropathy among agricultural and forestry workers, despite widespread 2,4-D use, the presence of antecedent illnesses not knowingly associated with 2,4-D poisoning in individuals presenting with peripheral polyneuropathy and the fact that symptoms developed after most of the 2,4-D would have cleared the body, provide strong evidence that the polyneuropathies described in the case reports were unlikely to be associated with 2,4-D exposure. Rather, the symptoms are more characteristic of conditions having a multifocal etiology.

Peripheral neuropathy has not been observed in experimental animals even at high, sustained doses of 2,4-D although doses in excess of 50 to 100 mg/kg have been associated with neurotoxicity as manifested by skeletal muscle myotonia, changes in neurotransmitter concentrations in the brain accompanied by behavioral changes, and damage to the blood/brain barrier. These effects occur only at doses which exceed the threshold for saturation of renal tubular secretion which results in decreased capacity for excretion of 2,4-D and, hence, prolonged and higher systemic exposure than would occur at doses levels at which maximal excretion occurs (*i.e.*, below 30 to 50 mg/kg body weight/day). Neurotoxic effects do not occur in laboratory animals exposed to 2,4-D at doses below the threshold

for saturation of renal tubular secretion, and would not be expected to occur in humans exposed below this threshold. The following is a review and critique of the studies relating neurotoxicity to 2,4-D exposure.

There are several reports that have associated the development of psychological abnormalities and peripheral neuropathies with exposure to phenoxy herbicides. Several case-reports have described the development of a peripheral neuropathy, allegedly as a result of accidental acute exposure to 2,4-D esters, ammonium salts, and the parent acid (Goldstein *et al.*, 1959; Todd, 1962; Foissac-Gegoux *et al.*, 1962; Berkley and McGee, 1963; Fullerton *et al.*, 1969; Berwick, 1970; Brandt, 1971; Bezuglyi *et al.*, 1976; Fleck, 1985). In most of these case-reports, a syndrome characterized by reduced nerve conduction velocity, incomplete paralysis, abnormal tendon reflexes and sensory neuropathy was reported. In several cases, recovery was incomplete even several years after the exposure.

In a recent critical review, Mattsson and Eisenbrandt (1990) concluded that these case-reports of polyneuropathies in humans were unlikely to have been the result of 2,4-D exposure. This conclusion was based on several lines of evidence. First, given the widespread use of 2,4-D in agriculture and forestry, they concluded that there were far too few cases of polyneuropathy reported in the scientific literature for a plausible cause-and-effect relationship to exist. In fact the vast majority of individuals who have been exposed to large doses of various 2,4-D formulations have not shown signs of peripheral neuropathy. Secondly, in several of the reported cases, patients reported symptoms such as fever, gastrointestinal distress, and swelling of the extremities prior to the development of symptoms that were suggestive of peripheral polyneuropathy. According to them, these antecedent illnesses, which have not generally been reported in most other cases of 2,4-D over-exposure, are suggestive of a multifocal etiology for the reported polyneuropathies. Thirdly, the time sequence of the illnesses reported in the case studies was not considered by them to be in accord with the known pharmacokinetics of 2,4-D (*e.g.*, 2,4-D has a plasma clearance half-life of about seven to 16 hours). The onset of the symptoms in the reported cases occurred when most of the 2,4-D would have cleared the body. Finally, they cited several experimental animal studies (Mattsson *et al.*, 1986a,b; Gorzinski *et al.*, 1987; Steiss *et al.*, 1987) which have reported that there is no evidence of 2,4-D induced peripheral neuropathy, even when animals were administered doses that were many times greater than those experienced by the most heavily exposed humans.

One study which has been cited as supporting evidence for 2,4-D induced polyneuropathy is the report by Singer *et al.* (1982) that sural nerve conduction velocities were significantly reduced and correlated to length of employment in workers at a facility manufacturing 2,4-D and 2,4,5-T. In this study however, as discussed by Mattsson and Eisenbrandt (1990), the actual relationship between slow nerve conduction velocity and exposure to 2,4-D was unknown. In addition, no cases of peripheral polyneuropathy were reported. Also, the results of the Singer *et al.* (1982) study were not conclusive since the control subjects were not concurrent, were not from similar occupations, and were not tested under the same conditions. As a result, the Singer *et al.* (1982) study provides no definitive evidence that 2,4-D is causally related to the development of peripheral polyneuropathy in humans.

Based on the evidence presented in the critical review by Mattsson and Eisenbrandt (1990), it seems highly unlikely that the few reported cases of polyneuropathy in agricultural/forestry workers are causally related to exposure to 2,4-D. In addition, 2,4-D exposure does not appear to be related to the development of other neurological disorders such as Parkinson's disease (Hertzman, 1990).

Animal studies have demonstrated that oral and dermal exposure to various 2,4-D acids, esters and salts does not result in peripheral polyneuropathy; however the data indicate that high-dose (generally 50 to 500 mg/kg body weight) acute exposure to various 2,4-D formulations can result in:

1) skeletal muscle myotonia (Kwiecinski, 1981; Steiss *et al.*, 1987; Elo and MacDonald, 1989; Arnold *et al.*, 1991; Beasley *et al.*, 1991),
2) alterations to the concentrations of neurotransmitters in the brain and/or behavioral changes (Podolak, 1981a,b; Schulze, 1988; Schulze and Dougherty, 1988; Elo and MacDonald, 1989; de Duffard *et al.*, 1990a,b),
3) damage to the blood/brain barrier, and
4) alterations in the organic ion transport system (Pritchard, 1980; Hervonen *et al.*, 1982; Kim *et al.*, 1983; Tyynela *et al.*, 1986; Elo *et al.*, 1988; Kim *et al.*, 1988; Tyynela *et al.*, 1990).

These effects all occur at exposure levels in excess of the threshold for saturation of renal tubular secretion, and cannot be considered relevant to humans exposed at doses below those associated with non-linear pharmacokinetics.

Myotonia (probably in humans) resulting from 2,4-D exposure has been reported in an abstract (Buslovich and Pichugin, 1983). These authors reported myotonia-specific and non-specific effects in the electromyographic recordings taken from patients suffering from acute 2,4-D poisoning. Recent animal studies have verified that 2,4-D exposure can induce abnormalities detectable in electromyogram recordings. Studies in which dogs were administered a single peroral dose of 2,4-D amine or acid indicate that the NOEL for the development of clinical myotonia in this species lies between 50 and 100 mg/kg (Steiss *et al.*, 1987; Arnold *et al.*, 1991; Beasley *et al.*, 1991).

Subclinical myotonia, noted in electromyogram recordings, has been observed in dogs treated with single oral doses in the range of 10 to 50 mg/kg (Steiss *et al.*, 1987; Arnold *et al.*, 1991; Beasley *et al.*, 1991). The NOEL for the development of subclinical myotonia in dogs was determined to be approximately 1.3 mg/kg body weight (Beasley *et al.*, 1991). Abnormalities in the electromyogram recordings in the dog treated at the next highest dose level of 8.8 mg/kg body weight were of a very mild nature and only occurred in response to electrode insertion in three of the eight muscle groups tested. Based on the mild nature of the effects observed, the 8.8 mg/kg body weight dose level may be considered a NOAEL. The NOELs and the NOAEL for the development of clinical and subclinical myotonia in dogs are not conclusive since only one animal per dose level was used in two dog studies conducted by Arnold *et al.* (1991) and Beasley *et al.* (1991). An acute dog study conducted by Steiss *et al.* (1987), in which four animals were included in each dose group (0, 25, 50, 75, 100, and 125 mg/kg body weight), indicated a NOEL of 25 mg/kg body weight for 2,4-D-induced myotonia based on the absence of both clinical myotonia and abnormalities in the electromyogram recording. Since the Steiss *et al.* (1987) study utilized a greater number of animals than either the Beasley *et al.* (1991) or the Arnold *et al.* (1991) studies (four/dose group versus only one/dose group), the NOEL of 25 mg/kg body weight for 2,4-D-induced myotonia was considered to be more reliable than the NOELs/NOAEL determined from the other two studies.

Studies with rats have indicated that 2,4-D acid administered via gavage at doses of 20 to 80 mg/kg body weight/treatment, twice weekly for five weeks, can increase hindlimb and forelimb grip strength; a condition suggestive of myotonia (Squibb *et al.*, 1983). Myotonia induced by 2,4-D does not appear to be the result of toxicological action upon the central nervous system (Buslovich and Pichugin, 1983), but appears to be due to effects mediated at the junction of skeletal muscle nerves and muscle tissue. The biochemical mechanism involved in the induction of clinical and subclinical myotonia in

experimental animals is not well understood; however, according to Rudel and Senges (1972) alteration of chloride ion conductance in muscle fibers appears to be involved. The development of myotonia in animals exposed to high doses of 2,4-D is not indicative of potential to induce peripheral polyneuropathy in humans since the induction of myotonia in animals was not accompanied by any pathological effects on the nervous system.

In the central nervous system *per se*, neurobehavioral changes, damage to the blood/brain barrier, alterations to membrane-related organic ion transport systems and alterations to the concentrations and turnover rates of several neurotransmitters and neurotransmitter metabolites in the brains of rats and mice have resulted from oral, subcutaneous and intraperitoneal exposures to various 2,4-D formulations, but only at dose levels which produced other overt signs of toxicity (*e.g.*, myotonia and lethargy).

In acute studies conducted using beagle dogs, clinical signs of central nervous system depression and/or abnormalities in the electroencephalogram (EEG) were only observed when 2,4-D was administered at doses of 175 mg/kg or greater (Arnold *et al.*, 1991). Associated with these observations were 2,4-D serum concentrations in excess of 1000 ppm and urinary 2,4-D concentrations of 200 ppm or greater. The doses associated with EEG abnormalities cannot be stated with certainty since the studies in which EEG monitoring was conducted utilized only one animal per dose level. In addition, some of the lowest dose animals were used in more than one study, raising questions as to their clinical integrity.

In rats, high-dose exposure to 2,4-D has been reported to induce behavioral changes (de Duffard *et al.*, 1990b). de Duffard *et al.* (1990b) reported that Wistar rats exposed via the diet for 15 or 17 days to 69 mg 2,4-D n-butyl ester/kg body weight/day displayed poorer scores in tests of active avoidance learning as well as in rotarod and open field tests. The results in these particular behavior tests, however, were not consistent for animals of differing sexual status (*e.g.*, males, castrated males, nulliparous and pregnant females). The variability in the results of the de Duffard *et al.* (1990b) experiment preclude any meaningful interpretation of the data.

Male and female rats treated with 69 mg 2,4-D n-butyl ester/kg body weight/day for either 15 or 45 days displayed alterations in concentrations of 5-hydroxytryptamine (5-HT) and 5-hydroxyindolacetic acid (5-HIAA) in the brain (de Duffard *et al.*, 1990a). Concentrations of 5-HIAA in the brain were

also significantly increased in Wistar rats injected with a single 200 mg/kg subcutaneous dose of 2,4-D sodium salt (Elo and MacDonald, 1989). The concentrations of 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were also increased in the cerebral-spinal fluid of rats treated with 2,4-D sodium salt at dose levels of 100 mg/kg and to a much lesser extent at 30 mg/kg. Based on data reported by two independent groups of researchers (Elo and MacDonald, 1989; de Duffard *et al.*, 1990a) the NOEL for acute, 2,4-D-induced alterations to the concentrations of neurotransmitters and associated metabolites in the central nervous system (CNS) of rats appeared to be approximately 10 to 30 mg/kg body weight.

Several biochemical and physiological effects have also been observed which may account for the neurochemical alterations reported to occur in response to acute or subacute, high-dose (*i.e.*, approximately 50 to 100 mg/kg) exposures to 2,4-D. First, there is evidence that the blood/brain barrier, responsible for controlling the permeability of the brain cells to xenobiotic substances, may be compromised by high, toxic doses of 2,4-D (Tyynela *et al.*, 1986; Elo *et al.*, 1988). Certain areas of the medulla oblongata and the cerebral cortex of rats, acutely dosed with 2,4-D acid via stomach tube at doses of 300 mg/kg body weight or more, were found to show extensive staining after intravenous treatment with Evans blue to highlight areas of extravasation of albumin (Elo *et al.*, 1988). The effect of 2,4-D treatment on albumin permeation of the CNS (indicative of damage to the blood/brain barrier) was more extensive than that observed with a similar dose of 2-methyl-4-chlorophenoxyacetic acid.

No apparent damage to the blood/brain barrier was observed with a single 150 mg/kg dose of 2,4-D acid. Similar observations regarding the blood/brain barrier-damaging effects of high dose exposure to 2,4-D in rats were reported in a study by Tyynela *et al.* (1990). Damage to the blood/brain barrier could result in increased entry of 2,4-D into the brain or could retard the elimination of 2,4-D from the brain, leading to an accumulation of this compound in brain tissue.

Elo and Ylitalo (1979) have reported that the concentrations of 2,4-D in the brain rose rapidly when 2,4-D was administered at doses of between 100 and 250 mg/kg body weight. When radiolabeled 2,4-D sodium salt was administered via intraperitoneal injection to rats pretreated with a 250 mg/kg dose of unlabeled 2,4-D sodium salt at a dose of 20 to 50 mg/kg body weight, concentrations of 2,4-D in the brain and cerebrospinal fluid, respectively, were reported to be approximately seven- and 22-fold higher compared to the concentrations reported for rats which had not been pretreated with 250 mg

unlabeled 2,4-D/kg body weight (Elo and Ylitalo, 1979). Studies with the 2,4-D analogue, 2-methyl-4-chlorophenoxyacetic acid, suggest that the concentrations of 2,4-D in the brain increase significantly at doses of 100 mg/kg or greater (Elo and Ylitalo, 1977, 1979). Kim *et al.* (1988) determined that doses of 2,4-D as low as 40 mg/kg administered for two consecutive days to mice could induce an increased accumulation of this compound in the brain tissue relative to the plasma. In the Kim *et al.* (1988) study, it was also determined that the increased accumulation of 2,4-D in the brain at high doses was not likely the result of increased permeability of the blood/brain barrier since the entry of the organic solute, 2-deoxyglucose, into rabbit brain was unaffected by 2,4-D pretreatment. Kim *et al.* (1988) indicated that reduced elimination of 2,4-D from the brain via the choroid plexus was likely responsible for the increased accumulation of 2,4-D.

The mechanism by which 2,4-D accumulates in the brain when administered at doses of greater than 50 to 100 mg/kg body weight has been suggested to be through the inhibition of the organic acid transport pathway which actively eliminates acidic metabolites from the brain through the blood/brain barrier (Ylitalo *et al.*, 1990). This process is especially active in the choroid plexus. The inhibition of the transport of organic acids out of the brain at high doses correlates well with the results of the studies conducted by Elo and MacDonald (1989) and de Duffard *et al.* (1991) which have indicated that concentrations of acidic metabolites of neurotransmitters (*e.g.*, 5-HT, 5-HIAA, DOPAC, and HVA) are increased in the brains of rats treated with single administrations of 2,4-D at dose levels of 30 to 200 mg/kg body weight. No effect levels for increased concentrations of neurotransmitter metabolites in the brain ranged from 10 to 30 mg/kg body weight (Elo and MacDonald, 1989) to less than 69 mg/kg body weight (de Duffard *et al.*, 1990a).

Given that the doses of 2,4-D required to produce inhibition of the organic ion transportation pathway in the brains of experimental animals appear to be lower than those required to induce damage to the blood/brain barrier, it is likely that inhibition of organic ion transport is the most important mechanism by which administration of 2,4-D at high doses induces a threshold dependent increase in the concentrations of this compound in the brain. This hypothesis is in good agreement with the observations that high-dose 2,4-D exposure in rabbits had no effect on the rate at which 2-deoxyglucose permeated the blood/brain barrier (Kim *et al.*, 1988).

The dose-response relationship for the induction of central nervous system effects by 2,4-D is clearly of

a threshold nature, since no clinically observable adverse effects have been observed at doses below 10 to 30 mg/kg body weight even in long-term studies and the mechanism by which the effects occur at high doses appears to be dependent upon the inhibition of the organic acid transport system and/or damage to the blood/brain barrier. Exposures to 2,4-D below the threshold for inhibition of the organic acid transportation pathway (approximately 40 to 100 mg/kg body weight) are not associated with increased concentrations of 2,4-D in the brain, alterations to brain neurochemistry, behavioral effects, or central nervous system pathology.

No lesions of the CNS or overt clinical signs of CNS toxicity were observed in six subchronic toxicity tests in rats (ITF, 1991a,b,c; Dow *et al.*, 1991a,b,c) and one subchronic toxicity test in mice (ITF, 1991d) conducted using either 2,4-D acid, 2,4-D triisopropanolamine, 2,4-D butoxyethyl ester, 2,4-D-2-ethylhexyl ester, or 2,4-D isopropylamine at dose levels of up to 300 to 560 mg/kg body weight/day. All of the subchronic studies were of high quality and conducted according to accepted GLP guidelines. In several of the subchronic toxicity studies the relative brain weights of the treated animals were elevated in the highest and/or second-to-highest dose groups (four dose groups were used) when compared to controls (ITF, 1991a,c,d; Dow *et al.*, 1991a,b). This observation in all of these studies, however, was the result of statistically significant reductions in the absolute body weights of the high-dose animals in comparison to the controls. In addition, even though the dose levels utilized in some of these subchronic toxicity studies exceeded 500 mg/kg body weight/day, very few signs of muscular myotonia were reported. Only in the subchronic toxicity study on 2,4-D-2-ethylhexy ester were some of the high-dose animals reported to show clinical signs that could possibly be related to myotonia (*e.g.*, hunched posture, languid behavior, ataxia) (ITF, 1991b).

The lack of clinical effects in the subchronic toxicity studies that would be indicative of central nervous system toxicity or muscular myotonia is not in agreement with observations made by the authors of acute and subacute studies described earlier. This discrepancy may in part be due to the fact that the observations reported in the acute studies were concerned with neurotransmitter chemistry and biochemical mechanisms. These types of observations are not routinely studied in subchronic studies and were not investigated in the subchronic studies conducted on the various formulations of 2,4-D. Also, it may not be possible to directly compare the results obtained regarding the myotonic effects of 2,4-D in dogs to the observations made from studies in rodents. Another consideration that must be taken into account is that in the acute studies, bolus doses of 2,4-D in excess of 100 mg/kg body

weight were administered. On the other hand, in the subchronic studies, total daily intakes of 2,4-D were 300 mg/kg body weight or more in the highest dose groups. However, these particular animals may not have experienced the same peak serum, and therefore peak brain concentrations of 2,4-D, as the acutely exposed animals since the 2,4-D doses in the subchronic studies would have been spread out over the entire day or over the period when the animals were feeding. The rapid uptake and short elimination half-life of 2,4-D is likely to favor the production of higher serum and brain concentrations of 2,4-D when this compound is administered as a single bolus dose rather than as a daily dose.

Immunotoxicity studies

To date there is little evidence that 2,4-D induces immunotoxicity in exposed human populations. The hematopoietic system has been alleged to be a target organ for 2,4-D in some people (Young *et al.*, 1978). There have also been attempts to associate specific disease conditions with 2,4-D, agricultural practices, Vietnam or Agent Orange exposure (USAF, 1983; Stubbs *et al.*, 1984; Delzell and Grufferman, 1985; Thomas and Kang, 1990; Zumwalt, 1990). These particular epidemiology studies or reviews, however, a) found effects which were not statistically significant, b) examined populations which were concomitantly exposed to 2,4,5-T, T_4CDD and/or a variety of other herbicides and pesticides, and c) utilized exposed populations that were not likely to be representative of the general population (Vietnam veteran versus non-veteran and farmer versus non-farmer). In addition, at least two other epidemiology studies reported no immunological effects in other agricultural workers and Vietnam veterans (Stark *et al.*, 1987; Wolfe *et al.*, 1990). A series of annual reviews of the scientific literature on phenoxy herbicides commissioned by the U.S. Department of Veterans Affairs (formerly the Veterans Administration) has not found 2,4-D to be associated with immunological effects (JRB Associates, 1981; Clement Associates, 1984; 1985; 1986; 1987; 1988; 1989; 1990; 1991).

Two groups of researchers, one at the University of Saskatoon and one at the Institute of Immunology in Moscow have attempted to assess the potential effects of 2,4-D on various immune system functional parameters in mice (Blakley, 1986; Blakley and Blakley, 1986; Blakley and Schiefer, 1986; Zhamsaranova *et al.*, 1987). Several animal models have been used to evaluate the effects of acute and subacute oral and dermal exposures of 2,4-D (mostly the n-butyl ester) on the antibody response to sheep red blood cells (RBCs), the response of B- and T-lymphocytes to mitogenic stimuli and the development of sensitization and delayed hypersensitivity-type reactions (Cushman and Street, 1982;

Blakley, 1986; Blakley and Blakley, 1986; Blakley and Schiefer, 1986; Zhamsaranova *et al.*, 1987).

In summary, the epidemiological data do not suggest that 2,4-D exposure in humans can induce immunotoxicity. The data from the studies conducted by researchers at the University of Saskatoon and at the Institute of Immunology in Moscow were inadequate to properly assess the potential for 2,4-D to induce immunologic alterations or dysfunction in experimental animals. In general, these studies produced results of T- and B-cell mitogenesis rates that were inconsistent for different exposure routes (oral and dermal) and inconsistent for acute and subacute exposure conditions. Effects reported were generally not reproducible. In addition, the biological relevance of the results is difficult to ascertain given the lack of data provided in these studies regarding the normal range of values for the immunological parameters tested in the mice strain used. In chronic studies conducted according to GLP, no effects attributable to 2,4-D were observed at any dose level in the hematological parameters measured or upon histopathological examination of selected lymphoid tissues. Based on the available data, there is no significant evidence to indicate that 2,4-D has potential to induce immunological dysfunction, especially at the dose levels expected to be encountered under normal human exposure scenarios. While a weak sensitization response was observed in one study in which a 2,4-D protein conjugate was administered to mice via intraperitoneal injection, the significance of this is questionable given that dermal applications of 2,4-D to mice failed to induce any evidence of a hypersensitivity-type reaction.

Specific Studies

Table 8 lists the epidemiology studies pertinent to 2,4-D and immunotoxicity.

The baseline mortality study on United States Air Force members exposed to large quantities of Agent Orange during the Vietnam War, due to their participation in Operation Ranch Hand, found a statistically insignificant excess of digestive disorder deaths (USAF School of Aerospace Medicine, 1983). The most recent morbidity update on that cohort found no immunologic abnormalities (Wolfe *et al.*, 1990). Thomas and Kang (1990) reported a significant excess of digestive disorder diseases in United States Vietnam Army Chemical Corps veterans. This cohort was exposed to riot control substances and burning agents as well as herbicides, though, so the relevance to 2,4-D cannot be determined.

Three agricultural cohort studies were reviewed for this report, but their relevance to 2,4-D is unknown because no exposure data were presented, since farmers are exposed to numerous chemicals and infectious agents. Stubbs *et al.* (1984) reported that respiratory, infective and parasitic diseases were elevated in California farmers. Delzell and Grufferman (1985) reported increased risks of tuberculosis and diseases of the skin and subcutaneous tissue in North Carolina farmers. Stark *et al.* (1987), on the other hand, found fewer than expected deaths for each major category of deaths in New York farmers.

One research team reported that patch testing with 2,4-D produced allergic reactions in three of 30 farmers with contact dermatitis, but this contributes little knowledge to the discussion because it was a subjective case series (Sharma and Kaur, 1990).

The results of a number of subchronic and chronic toxicity tests conducted in experimental animals have indicated that 2,4-D exposure has no adverse effects on any serum or biochemical measure of immune status (*e.g.,* white blood cell count, hemoglobin, hematocrit, lymphocyte number, *etc.*). In addition, in these studies, the selected samples of lymphoid tissue which were collected for further examination showed no gross pathological or histological changes indicative of immune system toxicity.

In one experiment (Blakley, 1986), the effects of acute and subacute 2,4-D administration via stomach intubation to mice on B-cell immunoglobulin secretion and B- and T-cell proliferative responses to mitogens were investigated. Following the acute administration of 2,4-D n-butyl ester to groups of 10 DBF mice at doses of up to 200 mg/kg body weight, the antibody production towards sheep RBCs, as determined through the Jerne Plaque Assay, was considered by the authors to be increased in all treatment groups when compared to the antibody response to sheep RBCs by a group of 10 mice treated with the vehicle solution (sunflower seed oil) (Blakley, 1986). Although the number of antibody producing cells/10^6 spleen cells was increased in all treatment groups, no clear dose-response relationship was evident.

Contrary to the results obtained with the acute oral administration of 2,4-D, Blakley (1986) reported data which indicated that the number of antibody producing cells produced by groups of 10 female DBF mice administered either 0, 10, 50, or 100 mg/kg 2,4-D n-butyl ester three times per week for three weeks (Blakley, 1986) was unaffected by the 2,4-D treatment. Also, the number of antibody producing cells determined for the control mice in the subacute study appeared to be considerably higher than the values reported for the control mice of the same sex and strain in the acute study (*i.e.,* 689 ± 55 *versus* 487 ± 66 antibody producing cells/10^6 spleen cells). Given the wide variability in the antibody response between the acute and subacute control groups, along with the fact that no effect on antibody production was noted when 2,4-D n-butyl ester was administered for three weeks, the Blakley (1986) finding of a statistically significant effect of acute 2,4-D n-butyl ester treatment on antibody production may be considered to have little biological relevance and probably represents a spurious result.

The enhancing effect of an acute administration of 2,4-D to DBF mice on the antibody response towards sheep RBCs reported by Blakley (1986), was not replicated by Zhamsaranova *et al.* (1987) in

a similar study in which groups of CBA x C57Bl mice were orally exposed to 0, 4, 25, or 100 mg 2,4-D acid/kg body weight/day for either two or five days prior to and for two or five days after antigenic stimulation with sheep RBC. In contrast to the enhancement of the number of antibody producing cells reported in the acute study (Blakley, 1986), the data from the Zhamsaranova *et al.* (1987) study suggest that the number of antibody producing cells was reduced in response to 2,4-D treatment. Examination of the data indicated that the reduction in the antibody response was not dose-dependent. The Zhamsaranova *et al.* (1987) experiment was reported in an unedited Russian translation in which few details of the experimental protocol, animal handling and husbandry techniques were given. As a result, the Zhamsaranova *et al.* (1987) study was considered to be inadequate from which to draw conclusions concerning the potential effects of 2,4-D on the immune system.

Inconsistent and conflicting data regarding the effect of 2,4-D on the antibody production in response to antigenic challenge were also reported by Blakley and Blakley (1986) and Blakley and Schiefer (1986) in which a 2,4-D n-butyl ester solution was administered to CD-1 mice *in utero* (0 to 200 mg 2,4-D n-butyl ester/kg body weight) or via topical application (0 to 500 mg/kg in acute studies and 0 to 300 mg/kg body weight in subacute studies) to adult CD-1 mice. Acute dermal application was associated with decreased antibody production at the highest exposure levels, while no effects on antibody production occurred when 2,4-D was topically administered for three weeks or when mice were exposed *in utero* on day 11 of gestation. The lack of reproducibility of the observed antibody production responses, the wide variations observed in the control animal antibody responses, and the inconsistent and conflicting nature of the reported significant alterations to the antibody responses in the treated animals suggest that the effects on antibody production attributed by the two groups of researchers to 2,4-D n-butyl ester may, in fact, be spurious fluctuations and unrelated to 2,4-D exposure. In addition, none of the studies attempted to investigate the reversibility of the observed responses on antibody production after 2,4-D administration.

The response of the cell mediated immune system (associated with cytotoxic and other T-lymphocytes) to immunological challenge with known mitogens (inducers of B- or T-lymphocyte proliferative responses) in mice exposed to 2,4-D n-butyl ester or 2,4-D acid via gavage, topical administration, or *in utero*, has also been studied by the same researchers who attempted to elucidate the effects, if any, of 2,4-D administration on the humoral immune system (*e.g.*, antibody response to antigenic challenge) (Blakley, 1986; Blakley and Blakley, 1986; Blakley and Schiefer, 1986; Zhamsaranova *et al.*, 1987).

In the animal test systems used, the same as those described for the studies examining the effects of 2,4-D on the antibody production response, a single intragastric dose of 2,4-D n-butyl ester administered to DBF mice at dose levels ranging up to 200 mg/kg body weight was associated with a dose-dependent increase in B-lymphocyte proliferation in response to the B-cell mitogen *Escherichia coli* lipopolysaccharide (LPS) (Blakley, 1986). However, no effect on B-cell mitogenesis was reported when single doses of 2,4-D n-butyl ester ranging up to 500 mg/kg body weight were topically applied to CD-1 mice (Blakley and Schiefer, 1986). The results from the subacute treatment of mice with 2,4-D n-butyl ester also appeared to be conflicting. Treatment of female DBF mice via gastric intubation with 2,4-D n-butyl ester at doses of 10 to 50 mg/kg body weight, three times weekly, for three weeks induced an enhancement of the B-cell mitogenic response while an intragastric dose of 2,4-D of 100 mg/kg body weight induced a non-significant reduction in the B-cell proliferative response (Blakley, 1986). In the subacute dermal application study an enhanced, linear B-cell mitogenic response, that was not statistically significant, was observed (Blakley and Schiefer, 1986). CD-1 mice exposed to 2,4-D n-butyl ester *in utero* did not present any evidence of an enhanced B-cell proliferative response to the same B-cell mitogen (Blakley and Blakley, 1986).

The responses of the immune systems of DBA and CD-1 mice treated with 2,4-D n-butyl ester, via stomach intubation or dermal application, to the T-cell mitogen, Concanavalin A (Con A), in the studies conducted by the Blakley laboratory are also considered to be of an inconsistent and conflicting nature. According to Blakley (1986), 2,4-D n-butyl ester, administered intragastrically either as a single acute dose of up to 200 mg/kg body weight or as nine doses of up to 100 mg/kg body weight spread over three weeks, produced no statistically significant effect on the T-cell proliferative response. However, in CD-1 mice exposed *in utero* to pure 2,4-D n-butyl ester (single doses of up to 200 mg/kg body weight on day 11 of gestation), Blakley and Blakley (1986) observed that 2,4-D treatment was associated with a statistically non-significant reduction in the background rate of T-cell mitogenesis. In another study, in which 2,4-D was administered dermally to CD-1 mice (Blakley and Schiefer, 1986), the data indicated that there was no effect of 2,4-D treatment on T-cell mitogenesis after acute exposure; however, an increased T-cell mitogenic response appeared to occur after subacute dermal administration of 100 mg/kg body weight.

The reliability of the observations made, and the conclusions reached by Blakley and colleagues regarding the effects of 2,4-D treatment on T- and B-cell mitogenesis, must be questioned given that:

1) the responses taken as a whole from each of the individual studies were varied; showing increased, decreased and unchanged rates of mitogenesis in response to 2,4-D administration,

2) the alterations in the B- and T-cell proliferative responses were not consistent for different exposure routes (dermal and oral),

3) there were inconsistencies between the effects of acute and subacute 2,4-D treatment, and

4) the mitogenic responses to Con A and LPS in the mice strains used (DBF and CD-1) were not compared to any known range of values that would be expected.

These shortcomings, similar to those seen in the studies of antibody responses to sheep RBC from Blakley and colleagues (Blakley, 1986; Blakley and Blakley, 1986; Blakley and Schiefer, 1986) and Zhamsaranova *et al.* (1987), make any conclusions regarding the potential for 2,4-D to cause immunologic dysfunction questionable.

The capacity of 2,4-D to induce hypersensitivity/sensitization type responses has been evaluated by Cushman and Street (1982). In this particular study, the potential of 2,4-D (form not stated) to induce a sensitization reaction in mice after intraperitoneal injection with two doses of either 1, 10, or 100 μg/mouse was investigated using the passive cutaneous anaphylaxis test. Exposure of mice to 1 μg of a 2,4-D keyhole limpet protein conjugate was considered by the authors of the study to be sufficient to induce an increase in the IgE antibody titer associated with sensitization reactions. The antibody titers produced by challenge with 1 μg of 2,4-D-bovine serum albumin conjugate were well below the antibody titers produced by challenge with 1 μg of the positive control dinitrophenyl (*e.g.*, PCA titers of 16 to 64 *versus* 512 to 1024). Mice exposed to either 10 or 100 μg of 2,4-D conjugate were reported to have lower IgE antibody titers than those mice exposed to 1 μg. However, as cited by the authors of the experiment, this inverse dose relationship phenomena is also known to occur in other cases of chemically induced hypersensitivity. This being the result of the activation of T-suppressor cell

populations which attenuate the secretion of immunoglobulins (*e.g.,* IgE) by B-cells. This potential mechanism to explain Cushman and Street's (1982) observations, however, was not directly tested in their experiment.

The positive control which utilized dinitrophenyl as the challenge agent produced the expected high IgE titers following the second intraperitoneal injection. The authors considered that they ruled out the possibility that the conjugating protein used with the sensitizing doses of 2,4-D, keyhole limpet hemocyanin, could have been responsible for the increased IgE on the basis that bovine serum albumin was used as the conjugate when the challenge dose was applied. However, keyhole limpet hemocyanin is a known sensitizing antigen (Lefford, 1974; Holsapple *et al.,* 1984), and the possibility that common antigenic determinants exist between keyhole limpet hemocyanin and bovine serum albumin cannot be discounted.

When mice were exposed dermally to 2,4-D, the most probable route of exposure in occupationally exposed humans, Cushman and Street (1982) observed that the 2,4-D solution did not induce any increase in IgE antibody titres as observed when the 2,4-D was administered by intraperitoneal injection. The differences in the hypersensitivity responses between the intraperitoneal and the dermal routes of exposure were not likely the result of the failure of the animal model, since, according to the authors, dermal treatment with dinitrofluorobenzene (the positive control) induced a positive contact hypersensitivity response.

In summary, the experimental animal data, derived from two different groups of researchers (Blakley, 1986; Blakley and Blakley, 1986; Blakley and Schiefer, 1986; Zhamsaranova *et al.,* 1987) for direct T- and B-cell toxicity, were considered to be inadequate for the purposes of determining the immunotoxic potential of 2,4-D. In the studies examining the effects of acute and subacute 2,4-D exposure on the production of antibodies and proliferation of B- and T-cells, the results were generally inconsistent and not reproducible for differing exposure routes and regimens. In one study investigating the sensitization potential of 2,4-D, a weak sensitizing effect was observed when 2,4-D was administered via intraperitoneal injection; however, this could not be reproduced with dermal exposure (Cushman and Street, 1982). Chronic studies suggest that 2,4-D has no adverse effects on the immune system. Taken together, the experimental animal data do not indicate that 2,4-D has significant potential to induce immune system toxicity or dysfunction.

Reproductive toxicity studies

Some epidemiology studies have suggested a possible link between 2,4-D and reproductive or developmental effects; however, most of these studies lacked sufficient exposure information and were not adequate to derive any definitive conclusions. The effects on male reproductive capability reported in one such epidemiology study were not supported by results from well-conducted animal assays.

Several other reproductive and developmental studies have been conducted with 2,4-D or its derivatives in laboratory animals. Based on the available studies, it is evident that the NOEL for reproductive or developmental toxicity varies with species. The NOEL for developmental and/or reproductive toxicity appears to be 5, 40, and less than 90 mg/kg body weight/day in rats, hamsters, and rabbits, respectively.

At maternally toxic doses some teratogenic effects have been reported; however, these effects are likely related to toxicity and not considered to be relevant. At maternally non-toxic doses, 2,4-D and/or its derivatives did not show teratogenic effects in rats, hamsters, mice, and rabbits, but did show some potential for embryotoxicity and fetotoxicity. In rats, these effects were generally seen above the maximum tolerated dose (MTD) and therefore, may not be toxicologically significant. In the other species, MTD were not available. The studies conducted in mice are not entirely adequate for determining a NOEL. Studies by Courtney (1977) and Kavlock *et al.* (1987) indicated that there was some prenatal toxicity (*i.e.*, decreased fetal body weight) at doses of 87.5 mg/kg body weight/day or higher. Lower doses were not tested. The following review summarizes the critical studies on reproduction and developmental toxicity studies.

Overall, the results from the reproductive and developmental studies indicate that some effects may be attributable to 2,4-D exposures, but only at high levels above the MTD (*i.e.*, maternally toxic) or the saturation level for renal clearance. Lower levels of exposure, to which humans may be exposed, would not be expected to produce such effects.

Specific Studies

A number of human epidemiology studies have been conducted addressing the possible relationship between phenoxy acid herbicide exposure and reproductive effects. These studies are briefly summarized in Table 9. For the most part, inadequate exposure information in these studies renders them of limited value in assessing the potential of 2,4-D to cause reproductive dysfunction.

One study alleged an association between farmers' exposure to 2,4-D and the development of sperm abnormalities (Lerda and Rizzi, 1991). In this study, the authors compared the incidence of sperm abnormalities in 32 2,4-D-exposed farmers compared to a "matched" control population. The data from this study are uninterpretable for several reasons. No indication was given as to how the matched control population was selected. Moreover, the authors reported that the exposed population had, on average, 9.2 mg 2,4-D per liter of urine yet no data were presented on the true extent or duration of exposure. More importantly, while the authors stated that they had controlled for confounding factors, no evidence was provided as to how this was accomplished. Farmers are exposed to a multiplicity of chemicals, many of which could have accounted for the observed effects. Most importantly, the authors indicated that sperm volume and other measures were made within two hours of sample collection but no indication was given as to how samples were stored and handled during the analysis phase. Given the fragility of sperm, adherence to strictly controlled laboratory conditions is essential. Finally, the authors misquoted a reliable reference by indicating in their paper that Klaassen *et al.* (1986) reported that 2,4-D causes reproductive damage. Klaassen *et al.* (1986) make no such statement. Overall it may be concluded that the report of Lerda and Rizza (1991) contains too many technical problems to permit any conclusions to be drawn and their reported results are also not borne out by extensive reproductive studies conducted at very high doses of 2,4-D in rodents (see below) further de-emphasizing any importance that can be attached to this work (Johnson, personal communication).

Two further studies reporting male gonadal effects warrant some attention. The Industry Task Force subchronic dog study (ITF, 1990) reported testicular effects of 2,4-D, which at first glance might seem to support the findings reported by Lerda and Rizzi (1991). Close examination of the results of the dog study indicate however that any effects on the testes are confounded by the systemic toxicity reported for this study. In fact, at the 10 mg/kg dose, the only dose at which testicular effects were reported, two out of five male dogs had to be killed in moribund condition. All of the remaining three

dogs that completed the study showed evidence of systemic toxicity while only two showed reduced testes size coupled with slight to moderate hypospermia. These effects can easily be accounted for on the basis of systemic toxicity, stress, and changes in thyroid hormone status induced secondarily to 2,4-D toxicity.

A Russian study by Konstantinova and Shevelo (1986) reported a reduction in testes mass and sperm count coupled with variable changes in sperm motility in rats exposed to phenoxy herbicides. The authors divided groups of treated rats into those receiving "pure" 2,4-D, technical grade (42%) 2,4-5-T or a combination of the two. The results for testes appeared to be significant for 2,4-5-T and the combination of the two herbicides, while effects for 2,4-D were reported to be minimal. Since no control data were given on morphological or functional indices, it is impossible to interpret the significance of these findings. Certainly they are not in accord with the results of well-conducted 90-day studies employing U.S.-manufactured 2,4-D, which have shown no effects on testicular morphology. Overall, the available data indicate that 2,4-D, unless given at toxic doses, has no effects on the male reproductive indices.

The reproductive and developmental data available from rat species are mostly from single generation studies (Schwetz *et al.*, 1971; Khera and McKinley, 1972; Unger *et al.*, 1981; Mohammad and St. Omer, 1986; Chernoff *et al.*, 1990; ITF, 1992); however, two multi-generation studies have been conducted (Hansen *et al.*, 1971; ITF, 1992). In reviewing these studies, it was apparent that the maximum tolerated dose (MTD) of 2,4-D as the acid or esters (in acid equivalents) administered to pregnant rats was in the order of 87.5 mg/kg body weight/day. Therefore, in studies in which the rats were administered levels of 2,4-D greater than 87.5 mg/kg body weight/day, any effects reported in the embryos/fetuses may not be toxicologically significant. Moreover, the saturation level for renal clearance in rats is 50 mg/kg body weight/day, indicating that doses greater than this may saturate renal tubular secretion and result in decreased capacity for excretion of 2,4-D, prolonging systemic exposure.

The most common developmental effects reported in the rat studies included decreased fetal weight gain, increased incidence of lumbar ribs and wavy ribs, and delayed ossification of bone. In general, these effects did not show a clear dose-response, but had statistical significance at levels greater than or equal to the MTD of 87.5 mg/kg body weight/day. Cleft palate, the only true teratological effect reported, seen in the mouse studies, was evident only at high, maternally toxic doses and would not be

relevant to much lower levels of exposure expected with humans. This effect was not seen at similar doses in other species studied, including rabbits, rats, and hamsters.

In the first of the two multi-generation rat studies conducted, Hansen *et al.* (1971) administered up to 1500 ppm of 2,4-D (acid) in the diet (approximately 75 mg/kg body weight/day) to Osborne-Mendel rats for three consecutive generations. The only adverse effects reported were at the highest dose level and consisted of decreased body weights in weanlings and reduced survival of pups to 21 days. Fertility was not affected. Moreover, no reports of teratogenic effects were given. In addition, Hansen *et al.* (1971) tested the activity of liver aliesterase and liver acylamidase of both mitochondrial and microsomal fractions as well as whole liver homogenate from F_{2a} rats. No difference in activity between the F_{2a} generation and control rats was found.

In the second of the multigeneration studies, unpublished data from the Industry Task Force (ITF, 1992) indicated that administration of the highest dose level [80 mg 2,4-D (acid)/kg body weight/day] resulted in "excessive toxicity" in F_0 and F_1 rats and, as a result, the high dose group of the F_1 generation was culled. The toxicity reported included reduced maternal body weights and food consumption, decreased gestational length, decreased F_{1a} and F_{1b} body weights during lactation, reduced live litter sizes, and in the F_{1b} generation, excessive pup mortalities. In the available summary of this study, it was reported that the actual dose level administered as 80 mg/kg body weight/day might, in fact, have been higher. The next dose level of 20 mg/kg body weight/day resulted in virtually no adverse effects aside from a slight decrease in F_{1b} pup body weights during lactation and, again, it was reported in the summary that the actual dose level received by the rats may have been higher than 20 mg/kg body weight/day. Since the F_2 pups were necropsied and discarded, this effect could not be followed into the next generation. The Industry Task Force determined a NOEL of 5 mg/kg body weight/day for F_0 and F_1 generations based on these data.

The remaining single generation studies focused mainly on the embryotoxic, fetotoxic, and teratogenic potential of 2,4-D acid or its esters.

In one of the earlier studies by Schwetz *et al.* (1971), 2,4-D (acid) and its propylene glycol butyl ether (PGBE) and isooctyl (IO) esters were administered orally to pregnant Sprague-Dawley rats during gestation days six to 15. Treatment-related effects included reduced fetal body weight, delayed

ossification of bone, subcutaneous edema, and an increased incidence of lumbar and wavy ribs at doses of 50 to 87.5 mg 2,4-D molar equivalents/kg body weight/day. These effects were considered by the authors to indicate embryotoxicity and fetotoxicity, but not teratogenicity since there were no effects on fetal or neonatal development and survival. The data indicated that the NOEL for 2,4-D and the molar equivalents of its esters, PGBE and IO, was 25 mg/kg body weight/day. It should be noted that at the lower dose level of 12.5 mg PGBE (acid molar equivalent)/kg body weight/day, a statistically significant increase in delayed ossification of skull bone was reported; however, the authors indicated that this incidence was less than the spontaneous incidence in a second control group used for another part of the study.

Khera and McKinley (1972) conducted a study very similar to Schwetz *et al.* (1971) in which 2,4-D or its esters were administered to Wistar rats at doses up to 150 mg/kg body weight/day. At the highest dose levels (100 and 150 mg/kg body weight/day), there was a statistically significant increase in the frequency of skeletal defects including lumbar and wavy ribs, and fused sternae. At the two lower dose levels of 25 and 50 mg/kg body weight/day, malformations were reported; however, the significance was deemed small by probability testing. Minimal postnatal effects were reported for dose groups up to 50 mg/kg body weight/day. At higher levels, there was evidence of reduced viability, decreased litter sizes and reduced pup body weights.

In more recent work by Unger *et al.* (1981), who performed a similar study in CD® rats using the PGBE and IO esters of 2,4-D, an increased incidence of skeletal malformations (*i.e.*, lumbar ribs) was found in the high dose group (87.5 mg/kg body weight/day) for both esters. In the case of the IO ester, there were additional bone malformations including fused ribs, fused centri, and vertical fusion of vertebrae. Also, there was a statistically significant increase in the percentage of viable fetuses at a lower dose of 12.5 mg/kg body weight/day; however, this was not seen at any other dose level. Sporadic soft tissue anomalies were seen throughout all groups and showed no treatment-related consistencies. A NOEL of 25 mg/kg body weight/day was derived from these data.

Additional unpublished data from the Industry Task Force have shown similar results in CRI:CD BR VAF/Plus rats administered more than 50 mg 2,4-D DMA/kg body weight/day (ITF, 1992). Reported effects included reduced maternal body weight and food consumption. In addition, at the highest dose level, a decrease in fetal body weight and delayed ossification of bone were reported. The Industry

Task Force derived a maternal NOAEL of 12.5 mg/kg body weight/day and a developmental NOAEL of 50 mg/kg body weight/day from these data.

The potential developmental effect of 2,4-D on F344 rats also was studied by the Industry Task Force (ITF, 1992). No embryotoxic or teratogenic effects were reported at levels up to and including the highest dose level tested, 75 mg/kg body weight/day. Slight maternal toxicity in the form of reduced body weight was reported at 75 mg/kg body weight/day, but not at any of the lower dose levels studied (0 to 25 mg/kg body weight/day).

The single dose study by Chernoff (1990) also reported an increased incidence of supernumary ribs at the maternally toxic (*i.e.*, reduced maternal body weight) dose of 115 mg/kg body weight/day.

Mohammad and St. Omer (1986) conducted a study similar to those described previously, except the Sprague-Dawley rats were administered a 1:1 mixture of 2,4-D with 2,4,5-T (T_4CDD contamination of 0.0125 ppm). No malformations were reported at levels up to 125 mg/kg body weight/day. At the two highest dose levels (100 and 125 mg/kg body weight/day), there was a statistically significant decrease in maternal body weight and the number of live pups delivered. A reproduction/developmental NOEL of 50 mg/kg body weight/day was determined from these data.

Studies on the potential effect of 2,4-D on reproduction or neonatal development have also been conducted in mice and to some extent in rabbits and hamsters.

In the 1960s, Bionetics Research Laboratories (1968) studied the potential carcinogenic, teratogenic, and mutagenic activity of several pesticides including 2,4-D. The study was conducted in several strains of mice given subcutaneous injections of 2,4-D at doses ranging from 46 to 150 mg 2,4-D (or its ester)/kg body weight/day. The vehicle used was DMSO. For the most part, adverse effects were reported at dose levels greater than and including 100 mg 2,4-D equivalents/kg body weight/day. Most of the effects including defects such as microthalmia, agnathic, and anophthalmia were reported in one strain of mouse (BL-6). The reported effects were not consistent and possible maternal toxicity was not clearly reported. The route of administration (subcutaneous injection) also complicated interpretation of the results with respect to expected routes of exposure (*i.e.*, dermal, inhalation, and oral).

A more in-depth study was conducted by Courtney (1977) and involved the development of a prenatal development index. CD-1 mice were administered 2,4-D or its esters by gastric intubation during gestation days seven to 15 or for a fraction of that period. Cleft palate was reported for all the esters tested at the highest dose level (approximately 221 mg/kg body weight/day). The 2,4-D acid and its PGBE derivative also were reported to produce cleft palates at the lower dose tested (approximately 124 mg/kg body weight/day). No cleft palates were reported in the control mice. Decreased maternal body weight and relative liver weight were reported sporadically in the treated groups and did not show a clear dose-response. Fetal body weights were decreased significantly for all the treated groups excluding low dose *n*-butyl ester and the high dose 2,4-D acid in DMSO vehicle. A NOEL could not be derived from these data.

Most recently, Kavlock *et al.* (1987) administered a single dose (87.5 mg/kg body weight/day) of one of three 2,4-D species (acid, PGBE or IO esters) to pregnant CD-1 mice during gestation days eight to 12. The neonatal effects reported were limited to a statistically significant decrease in weight gain of fetuses measured on postnatal day one, but not on postnatal day three, when compared with controls. Data on maternal toxicity were not presented.

Additional studies in mice have been conducted with 2,4-D mixed with other pesticides such as 2,4,5-T or picloram (Båge *et al.*, 1973; Lamb *et al.*, 1981; Blakley *et al.*, 1989a,b,c).

Dose-dependent maternal toxicity was reported in the studies with 2,4-D and picloram at doses ranging from 0 to 336 mg 2,4-D/kg body weight/day plus 0 to 20 mg picloram/kg body weight/day. At the highest dose level, fetal effects included reduced number of live fetuses per litter and increased percentage of fetuses with developmental variants. Fetal body weight and crown-rump length were decreased whereas the percentage of abnormal and malformed fetuses (*i.e.*, cleft palate) was increased in a dose-dependent manner in the treated groups (Blakley *et al.*, 1989b).

In a second study, Blakley *et al.* (1989c) used lower dose levels and still reported similar findings at the highest dose level of 101.5 mg 2,4-D/kg body weight/day plus 6.1 mg picloram/kg body weight/day.

In another mixture study, this time with 2,4,5-T, effects such as fetal mortality, cleft palate, retarded fetal growth, skeletal malformations, and renal hemorrhage were reported at the highest subcutaneous dose administered (110 mg compound/kg body weight/day) of a 2:1 mixture of 2,4-D/2,4,5-T during gestation days six to 14 (Båge *et al.*, 1973).

No effect on reproduction or neonatal development from 2,4-D/2,4,5-T mixtures administered to male C57BL/6 mice were reported in a paternal exposure study at dose levels equalling 40 mg 2,4-D/kg body weight/day (Lamb *et al.*, 1981). At higher 2,4-D dose levels, however, Blakley *et al.* (1989a) reported an increase in mortality of treated male mice, an increase in pregnancy failure, and an increase in the percentage of malformed fetuses. This was statistically significant at a dose level of 157.6 mg 2,4-D/kg body weight/day plus 9.5 mg picloram/kg body weight/day, but not at the highest dose level tested of 247.3 mg 2,4-D/kg body weight/day plus 14.8 mg picloram/kg body weight/day.

The two other animal species studied were hamsters and rabbits. Only one study was available for review for each of these species.

Hamsters administered up to 100 mg 2,4-D/kg body weight/day by oral intubation during gestation days six to 10 showed no significant teratogenic effects. In two of the 2,4-D samples tested, a statistically significant increase in the percentage of fetal viability was reported at 40 mg/kg body weight/day for one sample and at the two highest doses (60 and 100 mg/kg body weight/day) for the second sample. The percentage of abnormalities per live litter tended to be slightly higher at the two highest dose levels, but the significance of this observation was not expanded upon in the study. Terata (*i.e.*, fused ribs) appeared sporadically in the treatment groups; however, the frequency was not significantly different from controls (Collins and Williams, 1971). A NOEL of 40 mg/kg body weight/day was derived from this study.

The Industry Task Force reported in unpublished data a teratology study with New Zealand White rabbits (ITF, 1992). The rabbits were administered 2,4-D as the acid by stomach tube during gestation days six to 18. At the highest dose level administered (90 mg/kg body weight/day), 2/20 does aborted, 2/20 does showed ataxia (one doe common to both effects), and maternal body weights were reduced. These effects were not evident at the lower dose levels (0 to 30 mg/kg body weight/day). No developmental effects on the fetus were reported at any dose level. The Industry Task Force derived a

maternal NOAEL of less than 90 mg/kg body weight/day and a developmental NOAEL of greater than 90 mg/kg body weight/day.

Some variation in toxicity occurred between 2,4-D and its esters as well as the animal species tested. For example, from the rat study by Schwetz *et al.* (1971), a potency order for 2,4-D and its esters was suggested to be as follows: IO > 2,4-D > PGBE. In contrast, using mouse data from the study by Courtney (1977), the following order was suggested: PGBE > 2,4-D > isopropyl > IO > *n*-butyl.

From the information presented above, it was apparent that some reproductive and developmental effects in laboratory animals may be attributed to exposure to high levels of 2,4-D during gestation. Many of the reported effects are at levels exceeding the MTD (*i.e.,* maternal toxicity) or the saturation level for renal clearance. The occasional effects seen at lower exposure levels were often sporadic, not dose-related, and not expected at the low levels to which humans may be exposed.

INTERPRETIVE DISCUSSION OF INFORMATION RELATING TO THE SAFETY OF 2,4-D

Previously, under Framework for evaluating the safety of 2,4-D, we outlined the method for the interpretive evaluation of the safety of 2,4-D. In the following section, information gleaned from this review of scientific data are placed into the context of the guiding principles outlined in Framework for evaluating the safety of 2,4-D.

Epidemiological considerations:

Avoidance of Bias in Selecting Populations to be Studied

The avoidance of selection bias is important from the perspective of both maintaining the ability to discern true relationships between independent and dependent variables and biasing influences that may be peculiar to a specific population. In addition, generalization of the results of a given study to populations beyond the study group is dependent on understanding the derivation of the study population. The key cohort studies in our analysis considered populations defined by job description. We saw little evidence that such an approach to defining study groups would introduce a serious

selection bias. Likewise, the key case-control studies considered drew both cases and controls from population-based registries. Such an approach is the most rigorous for this type of research. Overall, we judged the epidemiology studies considered to be well-designed from the perspective of avoiding selection bias.

Verification of the Exposure and the Outcome

Avoidance of biases possibly resulting from misclassification along both the independent (exposure) and dependent (outcome) variables are important interpretational parameters that should be considered in evaluating the epidemiology. Differential misclassification can lead to overestimation or underestimation of risk, depending on the direction of the misclassification. Non-differential misclassification is known to bias results toward the null. While the majority of the epidemiology studies considered were rigorous in defining and verifying the disease outcome variables, exposure information on individual study subjects was available in very few instances. For the most part, the 2,4-D exposure assessments upon which the epidemiology study results are based were deficient. As such, the possibility exists that the epidemiology study results have little direct bearing on the 2,4-D health effects question.

Use of an Appropriate Comparison Group

Overall, the key epidemiology studies considered in this review were rigorous in identifying appropriate reference populations and control groups.

Identification and Control of Confounding Factors

The epidemiology studies considered in this review were unable to identify and control the many potentially confounding influences inherent to studies of broad occupational categories. Multiple exposures characterized the workplaces and occupations studied, including chemical, biological and physical agents that could explain the associations observed in some studies. This problem is more cogent in situations like this where the observed associations are modest in terms of magnitude. The possibility exists that the apparent positive associations observed derived from uncontrolled confounding.

Magnitude of the Observed Association

The epidemiological studies considered in this review reported risk estimates that were very modest in terms of magnitude or size. For example, the overall risk estimates for the case-control studies of farmers conducted in Iowa, Minnesota, Kansas and Nebraska ranged from around 1.2, or a 20% increase in risk, to around 2.1, or just above a 100% increase or a doubling in risk. Because of selection, misclassification and confounding factors inherent to epidemiological research, scientists analyzing these types of data usually are skeptical of data showing less than a doubling in risk. Risks of small magnitude are possibly the result of uncontrolled biases.

Statistical Power to Identify a Health Risk

Overall, the studies reviewed were of modest statistical power. As such, the reports of no associations were most relevant to ruling out large risks associated with the exposures studied. Small risks may have been masked.

Whether the Exposure and the Outcome Occurred in the Proper Temporal Relationship

Temporal relationship and latency are important considerations in identifying causal associations (a cause must precede an effect by an appropriate amount of time). The key epidemiological studies reviewed appeared adequate in addressing this issue.

Whether Increases in Exposure Were Associated with Increases in the Outcome (Dose-Response)

A critical criterion for identifying a cause and effect relationship is the presence of a dose-response. In the majority of both the case control and the cohort studies reviewed, there was no evidence of a dose-response relationship between potential exposure to 2,4-D and any adverse health outcome. In the Kansas and Nebraska farmworker case-control studies, there was a suggestion of a dose-response, with risk of NHL increasing with number of days per year reported spraying herbicides. A possible dose-response for NHL and acres sprayed with herbicides was also reported in the farmer cohort study

by Wigle *et al*. (1990). We have reviewed these data and consider them tentative with respect to dose-response. First, because the exposure assessment in the Kansas and Nebraska studies was based primarily on recall of events 20 years in the past, and because many of the responses were from next of kin, a strong likelihood of misclassification along this variable exists. The exposure assessment by Wigle *et al*. also relied on self-reported exposure, although the farmers only had to remember back to the previous year. Second, the finding in the Kansas study was driven by seven cases of NHL with reported use of herbicides of more than 20 days per year; in the Nebraska study, the finding was driven by three such cases. Misclassification of one or two cases in these studies would cause the finding to disappear. Third, there is some doubt that use of 2,4-D at the high frequency of 20 days per year as reported in the Kansas and Nebraska studies is realistic for farmers. Finally, Wigle *et al*. also reported a significant dose-response between NHL and purchases of fuel and oil for farming, so there appears to be something in those data besides 2,4-D that needs to be considered. For these reasons, we believe that there is little evidence of a dose-response in these data.

Specificity of the Association Between Exposure and Effect

In the epidemiology studies reviewed, there was little evidence to suggest a specific relationship between exposure to 2,4-D and any health outcome. The most persistent hypothesis regarding 2,4-D and health effects is that addressing NHL following exposure. However, the data are weak with respect to this association, and it is possible that the apparent specificity is simply a function of the persistence of the hypothesis.

Consistency of the Findings Within a Given Study

Overall, no study reviewed showed internal consistency with respect to the possible relationship between 2,4-D and disease. The Kansas farmworker study, for example, suggested an association between days per year of use of 2,4-D and NHL; however, there was no association in the data for other surrogates for exposure intensity, such as number of years of use. The lack of consistency within these studies suggests that the reports of associations may be artifactual.

Consistency of Results Between Epidemiological Studies

The need for scientific findings to be replicated is a universal tenet. The epidemiological studies considered in this review are characterized by inconsistency in findings.

Overall Conclusions From Epidemiology

Although we reviewed over 100 reports of epidemiological studies conducted that arguably bear on the question of 2,4-D and health effects, few studies had sufficient rigor in the assessment of exposure to provide meaningful information. The persistent hypothesis that 2,4-D is a cause of NHL is not supported by the weight of the epidemiological evidence. The studies overall lend virtually no support to the thesis that a large or moderate cancer risk is associated with exposure to 2,4-D. As other reviews of the epidemiology of 2,4-D-related effects have concluded, our review indicates that the hypothesis of 2,4-D-induced human cancer is not supported strongly by the data and this link is far from established.

With respect to hypotheses regarding 2,4-D exposure and other health effects, such as immune system alteration, neurotoxicity and reproductive effects, the epidemiological studies do not provide evidence of any strong hypotheses regarding cause and effect.

Finally, and perhaps most importantly, the epidemiological data do not indicate that a measurable public health threat is presented by continued use of 2,4-D. Even if the 2,4-D-NHL hypothesis were assumed to be supported, the population attributable risk associated with 2,4-D use would be diminishingly small. With new label instructions and other safety precautions aimed at reducing exposures that have been adopted over the past few years, the potential public health impact of continued use of 2,4-D is likely to be so small as to be unmeasurable.

Toxicological considerations:

Biological Plausibility

2,4-D is a simple organic acid, with no structural similarity to known classes of chemical carcinogens. It is rapidly excreted, does not accumulate in the body, and is not metabolized to reactive

intermediates. The lack of biological reactivity of 2,4-D is consistent with the lack of genotoxic potential evident from numerous genotoxicity studies. Thus, there is no indication that 2,4-D could induce cancer through a genotoxic mechanism. Neither is there any basis for hypothesizing an alternative mechanism for carcinogenesis. 2,4-D has no known hormonal activity and does not induce target organ toxicity resulting in sustained cellular proliferation and proliferative changes associated with many epigenetic carcinogens. Given the lack of accumulation in any tissue and the rapid excretion (at doses which do not saturate renal clearance), it would not be expected that 2,4-D would cause specific target organ toxicity leading to tumorigenesis through an epigenetic mechanism. This is supported by the lack of significant target organ toxicity observed in chronic and subchronic studies in rodents at doses below the threshold for saturation of renal clearance. Although 2,4-D falls into a class of compounds causing hypolipidemia and hepatic peroxisome proliferation, these effects are only observed at high doses and are not associated with liver tumor induction in rats or mice. In addition to lack of classical carcinogenicity and lack of potential for carcinogenesis by an indirect mechanism, 2,4-D has shown no activity as a tumor promoter. Based on the wide body of mechanistic data on 2,4-D, there is no basis for the hypothesis of a plausible mechanism of carcinogenicity. The absence of animal carcinogenicity supports the absence of carcinogenic effects in humans.

Urinary excretion is the major elimination route following oral (Khanna and Fang, 1966; Pelletier *et al.*, 1989; Knopp and Schiller, 1992) or dermal (Knopp and Schiller, 1992; Moody *et al.*, 1990; 1991; Pelletier *et al.*, 1989) administration of 2,4-D. The primary mechanism of excretion is renal tubular secretion (Berndt and Koschier, 1973; Gehring and Betso, 1978; Orberg, 1980), an active process which becomes saturated in rats at doses in the 50 mg/kg body weight/day range (Khanna and Fang, 1966; Gorzinski *et al.*, 1987). Since plasma levels rise disproportionately with dose once saturation of renal tubular secretion occurs, any toxic effects observed only at saturating doses are not relevant in terms of expected effects in humans at lower doses which do not cause saturation. In humans (Feldman and Maibach, 1974; Kohli *et al.*, 1974; Sauerhoff *et al.*, 1977) and experimental animals (Khanna and Fang, 1966; Pelletier *et al.*, 1989; Knopp and Schiller, 1992) administered 2,4-D orally or by injection, 90 to 100% of the dose is recoverable in urine over the course of two to six days, indicating that measurement of 2,4-D in urine provides a reliable indicator of exposure. This is supported by the results of studies of workers occupationally exposed to 2,4-D, in which the amounts of 2,4-D excreted were directly related to estimates of exposure (Libich *et al.*, 1984; Grover *et al.*, 1986).

2,4-D is highly water soluble and does not accumulate in tissues at non-intoxicating exposure levels (Erne, 1966a; Clark *et al.*, 1975). Rising levels of 2,4-D in the brain relative to plasma of rats treated with intoxicating doses has been shown to be due to inhibition of active anion transport through the blood/brain barrier at high blood concentrations (Pritchard, 1980; Kim *et al.*, 1983; Tyynela *et al.*, 1990; Ylitalo *et al.*, 1990). Brain levels of 2,4-D are very low at doses which do not induce neurotoxic effects and there is no evidence that 2,4-D accumulates in any tissue below toxic doses (i.e., doses below 100 mg/kg body weight) (Elo and Ylitalo, 1979; Tyynela *et al.*, 1990).

A factor involved in the distribution of 2,4-D is the extent to which it binds to serum proteins (Erne, 1966b). 2,4-D is extensively bound to serum proteins at low plasma concentrations, and binding becomes saturated at higher concentrations (Orberg, 1980). As result of this phenomenon, the results of studies carried out at doses exceeding that which causes saturation of protein binding are not suitable for extrapolation to lower doses to which humans may be exposed. The affinity of 2,4-D for rat albumin is similar to its affinity for human albumin (Fang and Linstrom, 1980). This, along with similarities in the absorption and excretion of 2,4-D indicates that rats provide a good model for the study of pharmacokinetics of 2,4-D for the purpose of extrapolation to humans.

2,4-D is quickly absorbed and excreted, resulting in short half-lives for plasma clearance and urinary elimination. In human subjects given an oral dose of 5 mg/kg body weight, the half-life for plasma clearance ranged from seven to 16 hours, and the half-life for urinary elimination ranged from 10 to 28 hours (Sauerhoff *et al.*, 1977), in general agreement with kinetic parameters reported in a similar controlled study (Kohli *et al.*, 1974) and in studies of exposed workers (Nash *et al.*, 1982; Frank *et al.*, 1985). Since plasma and urinary clearance occurred according to first order kinetics, it can be assumed that 99% of the steady state would occur after seven half-lives under conditions of continuous exposure. That is, continuous oral dosing at 5 mg/kg body weight/day would result in steady state plasma levels developing within two to four days, with no long-term accumulation. In terms of plasma clearance and urinary excretion after the cessation of exposure, the plasma level of 2,4-D would decrease by 99% of the starting concentration within two to four days, and urinary excretion would be 99% complete within two to eight days. These estimates are expected to hold for dermal exposure as well, since it is assumed that in humans the skin would be washed within eight hours, thereby depleting the reservoir on the skin and diminishing the effect of this reservoir on the pharmacokinetics of 2,4-D

excretion. In agreement with this, a computer simulation of the body burden of 2,4-D resulting from repeated dermal exposure, assuming changing clothes and washing, predicted the maximum body burden would occur after four days and would amount to 0.7 times the daily absorbed dose (Ramsey *et al.*, 1980).

2,4-D is not metabolized to a reactive intermediate in animals or humans. In humans, 2,4-D itself, or acid hydrolyzable conjugates were excreted following ingestion of a 5 mg/kg body weight dose of 2,4-D acid (Kohli *et al.*, 1974; Sauerhoff *et al.*, 1977). Similarly in animals, only parent compound or acid hydrolyzable conjugates are excreted (Erne, 1966b; Grunow and Bohme, 1974; Clark *et al.*, 1975; Shulze *et al.*, 1985; Kelley and Vessey, 1987; Frantz and Kropscott, in press). The lack of evidence from metabolic studies for the generation of a reactive intermediate is consistent with the lack of genotoxic potential suggested from a battery of genotoxicity studies.

Overall the metabolism and pharmacokinetics data on 2,4-D indicate that it is rapidly absorbed and excreted, does not accumulate in any tissue at doses below those causing saturation of renal tubular secretion and is not metabolized to a reactive intermediate. This type of metabolic profile is not characteristic of a compound with tumorigenic potential. The lack of target organ accumulation, and lack of significant target organ toxicity in rodents rule out the suggestion of an epigenetic mechanism of tumorigenesis involving tissue damage and subsequent regenerative hyperproliferation. The lack of genotoxicity and lack of evidence for the formation of a reactive intermediate, as well as the rapid excretion of 2,4-D rule out the suggestion of a genotoxic mechanism. There is also no basis to suggest a hormonal mechanism of tumorigenesis. In completing the discussion of mechanisms of tumorigenesis which do not appear to be reasonably postulated for 2,4-D, one remains which must be explored in some detail. This is hepatic peroxisome proliferation leading to hepatocarcinogenesis. 2,4-D belongs to a class of structurally similar compounds which cause hypolipidemia and hepatic peroxisome proliferation in rodents (Vainio *et al.*, 1983; Lundgren *et al.*, 1987). The induction of peroxisome proliferation is known to be associated with the induction of hepatocellular tumors in rodents (Reddy *et al.*, 1980).

This class of compound includes some phenoxy herbicides such as lactoten (Butler *et al.*, 1988), hypolipidemic drugs (such as clofibrate) and industrial plasticizers (such as diethylhexylphthalate--DEHP) and its members cause a characteristic group of effects including: hepatomegaly, peroxisome

proliferation, induction of hepatic P450 and enzymes involved in fatty acid oxidation and hypolipidemia. These are all effects that were observed in rats treated with high doses of 2,4-D. It has been suggested that compounds showing these effects which are not genotoxic and are hepatocarcinogenic in rodent bioassays may induce tumors indirectly as a consequence of increased activity of flavin-dependent oxidases leading to increased production of hydrogen peroxide and other active oxygen species which may cause DNA damage (Cohen and Grasso, 1981; Reddy and Lalwani, 1983; Reddy and Rao, 1986; Rao and Reddy, 1987). A comparison of the biological activities of a number of hypolipidemic agents revealed that the magnitude of induction of hepatic hyperplasia correlated with tumor potency to the same or to a greater degree than did the magnitude of peroxisomal enzyme induction (Butterworth *et al.*, 1987). This supports the contention that cell proliferation may also play a role in the development of peroxisome proliferator-induced hepatocarcinogenesis in rodents (Butterworth *et al.*, 1987; Michalopoulos *et al.*, 1987; Conway *et al.*, 1989). A strong association has been shown for the induction of cell proliferation and chemical carcinogenesis in the liver. It is generally accepted that increasing the rate of cell turnover also increases the chances for DNA replication error to occur and become permanent. Further, DEHP and other peroxisome proliferators have been observed to stimulate cell division in rodent liver under certain experimental conditions (Ward *et al.*, 1986; Bieri *et al.*, 1988; Marsman *et al.*, 1988). Although the mechanism of hepatic tumorigenesis by hypolipidemic agents has yet to be fully elucidated, the available data clearly support the contention that the mechanism is indirect, with tumor development occurring only in association with dosing regimes causing prolonged occurrence of peroxisome proliferation and hepatomegaly (Reddy and Qureshi, 1979; Reddy *et al.*, 1980).

In the case of 2,4-D there has been no indication of hepatocarcinogenic potential from chronic toxicity studies in rats or mice (see Pharmacokinetics), or in a promotion assay employing rats pretreated with initiating doses of liver carcinogens (Abdellatif *et al.*, 1990). Changes associated with peroxisome proliferation, including increases in liver weight and effects on lipid metabolism, were not observed in rats (ITF, 1986) or mice (ITF, 1987) treated at doses of up to 45 mg/kg body weight/day, indicating that this dose level was below the threshold for these effects. Peroxisome proliferation was slightly increased in rats treated at doses of 100 mg/kg body weight/day ($p<0.05$) and was more than double control values at doses of 150 to 200 mg/kg body weight/day (Vainio *et al.*, 1983). In mice, peroxisome proliferation was induced at a dose of 100 mg/kg body weight/day (Lundgren *et al.*, 1987). The lack of hepatocarcinogenicity in rats and mice suggests that the small increase in

peroxisome proliferation observed in rodents treated with elevated doses of 2,4-D is not a biologically significant phenomenon.

The toxicological significance to humans of the hepatic response to hypolipidemic agents in rodents has been discussed in a number of reviews, with the general consensus being that due to the correlation between hepatocarcinogenicity and peroxisome proliferating activity in rodents, a lack of peroxisome proliferating activity in humans may suggest a lower or absent risk of liver cancer in man (Cohen and Grasso, 1981; de la Iglesia and Farber, 1982; Reddy and Lalwani, 1983; Monro, 1992).

Based on this evaluation of the mechanistic data on 2,4-D, it is clear that there are no known mechanisms of tumorigenesis that can be reasonably postulated, and that the characteristics of 2,4-D are consistent with those of non-carcinogenic compounds.

Consistency of the Results Between Disciplines

Various epidemiology studies have indicated that 2,4-D may be implicated in neurological, immunological, reproductive, and developmental effects. In general, these epidemiology studies could not thoroughly assess exposure and no definitive conclusions could be drawn specifically related to 2,4-D. Furthermore, any effects that were implicated in the epidemiology studies were not supported by studies in experimental animals. The results of the acute toxicity tests are tabulated in Appendix C, and show that 2,4-D has low acute toxicity in laboratory animals. Subchronic and chronic studies have shown that the two main target organs for 2,4-D toxicity are the kidney and thyroid. The NOEL in rats and mice was determined to be 15 mg 2,4-D/kg body weight/day, whereas in dogs it was 1 mg 2,4-D/kg body weight/day. In neurotoxicity studies, only overt myotonia has been verified in experimental animals, and only at doses which would result in systemic toxicity or saturation of the renal clearance mechanism. Various animal models have been used to evaluate the effects of 2,4-D on the immune system, but were inadequate to derive any conclusions. Any developmental or reproductive effects were only observed at maternally toxic doses or at renal clearance saturation levels. Overall, the levels of 2,4-D to which humans would be exposed under normal operating conditions would not be expected to result in any immunological, neurological, reproductive, or developmental effects.

Case-control studies suggesting positive associations between high frequency use of herbicides and NHL, the most persistent of the cancer hypotheses, are equivocal in that exposure to 2,4-D was inferred from self-reporting or next-of-kin reporting and not directly measured, point estimates of risk are low, and findings are based on very small numbers of cases in the various exposure subgroups. The exposures reported in these studies were likely to be very low and usually mixed chemical exposures, with those in the highest exposure groups receiving estimated annual doses of only 120 μg 2,4-D/kg body weight. Only an extremely potent carcinogen, more potent than any carcinogen yet identified, would be capable of inducing tumorigenesis at such a low dose, and as such would be expected to produce tumors in animals treated at doses orders of magnitude higher. This has not proven to be the case.

The hypothesis that 2,4-D causes cancer in humans is not supported by observed target tissue effects in animals. Animal studies of immune system toxicity and carcinogenicity also do not add support to the hypothesis. In addition, the epidemiology of NHL suggests that viruses and immune system modulation are risk factors, and these potential confounders have not been adequately controlled in the existing epidemiology studies of herbicide use.

Conclusions

In conclusion, the following key points have formed the framework for the weight-of-evidence analysis of the potential carcinogenicity of 2,4-D:

1) 2,4-D is rapidly excreted, is not metabolized to reactive intermediates, does not possess chemical characteristics associated with biological reactivity and has not produced genotoxic activity in animal systems;

2) 2,4-D does not demonstrate the biological actions that have been associated with known genotoxic or epigenetic carcinogens, or with tumor promoters;

3) high quality animal bioassays do not provide evidence that 2,4-D is an animal carcinogen, even at doses 5000 times in excess of the maximum to which humans may be exposed when wearing

recommended protective clothing;

4) the mechanistic and empirical evidence from studies using laboratory animals do not support the conclusion that 2,4-D would be a human carcinogen;

5) epidemiology studies have provided conflicting information on a possible association between 2,4-D exposure and human cancer;

6) the findings among epidemiology studies where exposure to 2,4-D is one of many chemical exposures being measured are inconsistent; this inconsistency could indicate that evidence for a causal association between 2,4-D and cancer is weak;

7) the toxicological data on 2,4-D and the immune system are inconsistent, weakening support for the hypothesis that 2,4-D is associated with the development of lymphatic tumors;

8) likely 2,4-D exposures received by humans in studies suggesting a positive association with cancer would be extremely low, implying that 2,4-D would have a degree of carcinogenic potency much higher than any other compound studied to date; furthermore, high risks have been reported in epidemiology studies where exposures were low, such as Hardell's work, while people who must have had much heavier exposure as sprayers or manufacturers have shown little or no increase in risk;

9) the hypothesis that 2,4-D causes NHL in humans, the most persistent of the 2,4-D cancer hypotheses, is not supported by observed target organ effects in animals;

10) epidemiology studies of 2,4-D and NHL have not adequately controlled for the potential confounding effects of viral and genetic risk factors, although such factors do not rule out chemical involvement;

11) although STS is particularly problematic to study due to diagnostic difficulties (Lynge *et al.*,

1987) the only two epidemiological studies[9] since Hardell's work to indicate a possible association between 2,4-D and STS (Saracci *et al.*, 1991; Hansen *et al.*, 1992) studied workers exposed to multiple herbicides, insecticides, fungicides, chlorophenols, and contaminants, making it difficult to implicate 2,4-D.

This review presents an integrated assessment of the public health impact of 2,4-D. Although some epidemiology studies have indicated a possible association between the use of phenoxy herbicides and NHL or STS in humans, some reviewers, including one of the peer reviewers of this document, believe that the inability to accurately assess exposure in these studies precludes considering these data, which could be the result of differential recollection rather than differential exposure. The results of experimental animal studies do not support the contention that 2,4-D is carcinogenic. Human exposure to 2,4-D occurs through repeated subchronic exposure periods reflective of its use pattern, not as continuous chronic exposure as was tested in experimental animals. The highest subchronic doses received by humans are much lower than the doses tested in chronic studies in experimental animals without evidence of carcinogenicity. Mechanistic studies provide evidence against all the known mechanisms of chemical carcinogenesis. Thus, 2,4-D would have to be an extremely potent carcinogen acting by a unique mechanism in order to cause cancer in humans at the doses, and under the chronic exposure scenarios, experienced by workers. The existence of such a potent human carcinogen that is not also an animal carcinogen would not be expected in light of the existing body of scientific evidence indicating that human carcinogens are also animal carcinogens. Importantly as well, agricultural practices suggest that herbicides are used in combination and that 2,4-D is rarely, if ever, used by itself. Therefore, any reported effects in these mixed exposure epidemiological studies cannot be attributed solely to 2,4-D. In addition, the majority of the epidemiological studies are based upon historical exposures involving less rigorous standards of application and protective clothing than the present or proposed practices. Actual exposures to 2,4-D today would be expected to be very limited. When viewed in its entirety, the data on 2,4-D indicate that the potential public health impact of 2,4-D, including the risk of human cancer, was negligible in the past and would be expected to be even smaller in the present and future under the proposed label directions.

[9]One Ranch Hand has died of STS (Michalek *et al.*, 1990), but this is too few to analyze.

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APPENDIX A

HUMAN EXPOSURE TO 2,4-D WHERE THE RECEPTOR WORE APPROPRIATE PROTECTIVE APPAREL THAT INCLUDED: RUBBER GLOVES; BOOTS MADE OF RUBBER OR OTHER MATERIALS OF LOW CHEMICAL PERMEABILITY; AND, COVERALLS

Exposure Conditions	Number of Subjects in Study	Measured Dose (internal dose unless otherwise specified)	Average Estimated Internal Dose	Exposure (number of days / year)	Reference
HOME GARDEN USERS					
Canadian home gardeners following application of 395g Weed-and-Feed 2,4-D under conditions of protective application.	11	Based on urinary excretion, exposure was estimated to range from ND[a] to 108 µg / person / day with an average[b] of 9.8 µg / person / day.	Based on urinary excretion, exposure was estimated to be 0.140 µg / kg BW for a 70 kg individual.	1 day granular form application in spring, followed by 1 day liquid application in fall (see below).	Harris, *et al.* 1992. J. Environ. Sci. Health B27:23-38.
Canadian home gardeners following application of 107g liquid 2,4-D under conditions of protective application.	11	Based on urinary excretion, exposure was estimated to range from ND[a] to 63 µg / person / day with an average[c] of 9.2 µg / person / day.	Based on urinary excretion, exposure was estimated to be 0.131 µg / kg BW for a 70 kg individual.	1 day liquid application in fall, preceded by 1 day granular application in spring (see above).	Harris, *et al.* 1992. J. Environ. Sci. Health B27:23-38.

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Exposure Conditions	Number of Subjects in Study	Measured Dose (internal dose unless otherwise specified)	Average Estimated Internal Dose	Exposure (number of days / year)	Reference
BYSTANDERS					
Canadian bystander to home garden use following application of 395g Weed-and-Feed 2,4-D under conditions of protective application.	11	Based on urinary excretion, exposure was ND[a] for all subjects.	Based on urinary excretion, exposure was ND[a] for all subjects.	1 day granular form application in spring, followed by 1 day liquid application in fall (see below).	Harris, *et al.* 1992. J. Environ. Sci. Health B27:23-38.
Canadian bystander to home garden use following application of 107g liquid 2,4-D under conditions of protective application.	10	Based on urinary excretion, exposure was ND[a] for all subjects.	Based on urinary excretion, exposure was ND[a] for all subjects.	1 day liquid application in fall, preceded by 1 day granular application in spring (see above).	Harris, *et al.* 1992. J. Environ. Sci. Health B27:23-38.
Exposure to observers of aerial application crews in Washington and Oregon - following special precautions.	6[d]	not given	Based on urinary excretion, the authors estimated the average exposure to be 0.09 ± 0.23[e] µg / kg BW / day (no range was given).	1 day with a 5-day post-spray urine sampling period.	Lavy and Mattice, 1984. ACS Symp.238:319-330.

(continued)

FARM WORKERS					
Canadian farmers involved in spraying their fields with 2,4-D by ground rig (no protective clothing worn)[f].	8	Based on urinary excretion, exposure was estimated to range from 150 to 1053[g] µg / person / spray operation with an average of 480 µg / person / spray operation. The average total dose over the spray period was 1787.5 µg / person with a range of 470 to 6320 µg / person.	Based on urinary excretion, exposure was estimated by the authors to be 5.78 µg / kg BW / spray operation. The average total dose for the spray period was estimated by the authors to be 21.7 µg / kg BW.	1 to 7 spray operations spread over 1 to 17 days (spray period).	Grover *et al.*, 1986. Arch. Environ. Contam. Toxicol. 15:677-686.
COMMERCIAL APPLICATORS					
Exposure to lawn care specialists in Ann Arbor, Michigan - spray gun	8	not given	Based on urinary excretion, the author estimated the average exposure to be 0.35 ± 0.17[h] µg / kg BW / day during the spray period (no range given).	The author stated that the applicators had been spraying lawns for at least three weeks, 6 days / week.	Yeary, 1986. Appl. Ind. Hyg. (1) 3:119-121.
Exposure to lawn care specialists in Columbus, Ohio - spray gun	12	not given	Based on urinary excretion, the author estimated the average exposure to be 1.38 ± 0.5[h] µg / kg BW / day during the spray period (no range given).	The author stated that the applicators had been spraying lawns for at least three weeks, 6 days / week.	Yeary, 1986. Appl. Ind. Hyg. (1) 3:119-121.

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Exposure to lawn care specialists in Hartford, Conneticut - spray gun	13	not given	Based on urinary excretion, the author estimated the average exposure to be 3.2±1.0[h] µg / kg BW / day during the spray period (no range given).	The author stated that the applicators had been spraying lawns for at least three weeks, 6 days / week.	Yeary, 1986. Appl. Ind. Hyg. (1) 3:119-121.
Exposure to lawn care specialists in Flint, Michigan - spray gun	12	not given	Based on urinary excretion, the author estimated the average exposure to be 6.3±1.8[h] µg / kg BW / day during the spray period (no range given).	The author stated that the applicators had been spraying lawns for at least three weeks, 6 days / week.	Yeary, 1986. Appl. Ind. Hyg. (1) 3:119-121.
Exposure to lawn care specialists in Flint, Michigan - injection gun	12	not given	Based on urinary excretion, the author estimated the average exposure to be 2.5±1.5[h] µg / kg BW / day during the spray period (no range given).	The author stated that the applicators had been spraying lawns for at least three weeks, 6 days / week.	Yeary, 1986. Appl. Ind. Hyg. (1) 3:119-121.
FORESTRY WORKERS					
Exposure to mixers and loaders during an 11-day aerial application period in 1981 in Canada.	2	Authors estimated exposure for the 2 subjects to be 408 and 2535 µg / person for the 11-day period.	Average exposure was estimated to be 2.52 µg / kg BW for each day of the 11-day period.	11-day spray period with 9- and 7-day post-spray urine sampling for the 2 subjects, respectively.	Frank *et al.* 1985. Arch. Environ. Contam. Toxicol. 14:427-435.
Exposure to supervisor	1	Authors estimated exposure	The exposure was	11-day spray period with 8-day	Frank *et al.* 1985.

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during an 11-day aerial application period in 1981 in Canada.		to be 135 µg / person for the 11-day period.	estimated to be 0.22 µg / kg BW for each day of the 11-day period.	post-spray urine sampling.	Arch. Environ. Contam. Toxicol. 14:427-435.
Exposure to mixer and loader during an 18-day aerial application period in 1982 in Canada.	1	Authors estimated exposure to be 16,276 µg / person for the 18-day period.	The exposure was estimated to be 11.3 µg / kg BW for each day of the 18-day period.	18-day spray period with 13-day post-spray urine sampling.	Frank *et al.* 1985. Arch. Environ. Contam. Toxicol. 14:427-435.
Exposure to mixer and balloon man during an 18-day aerial application period in 1982 in Canada.	1	Authors estimated exposure to be 7,304 µg / person for the 18-day period.	The exposure was estimated to be 4.95 µg / kg BW for each day of the 18-day period.	18-day spray period with 10-day post-spray urine sampling.	Frank *et al.* 1985. Arch. Environ. Contam. Toxicol. 14:427-435.
Exposure to balloon man during an 18-day aerial application period in 1982 in Canada.	1	Authors estimated exposure to be 5,276 µg / person for the 18-day period	The exposure was estimated to be 3.91 µg / kg BW for each day of the 18-day period.	18-day spray period with 8-day post-spray urine sampling.	Frank *et al.* 1985. Arch. Environ. Contam. Toxicol. 14:427-435.
Exposure to backpack workers following special precautions in Arkansas, Oklahoma and Mississippi.	20	not given	Based on urinary excretion, exposure was estimated to range from 30.1 to 244 µg / kg BW / day with an average of 98 µg / kg BW / day.[i]	1-day spray period with 4-day post-spray urine sampling.	Lavy, *et al.* 1987. Environ. Toxicol. and Chem. 6:209-224.
Exposure to injection bar workers following special precautions in Arkansas,	20	not given	Based on urinary excretion, exposure was estimated to range from ND[j] to 12.1	1-day spray period with 4-day post-spray urine sampling.	Lavy, *et al.* 1987. Environ. Toxicol. and Chem. 6:209-224.

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Oklahoma and Mississippi.			µg / kg BW / day with an average of 4.3 µg / kg BW / day.[i]		
Exposure to hypohatchet workers following special precautions in Arkansas, Oklahoma and Mississippi.	20	not given	Based on urinary excretion, exposure was estimated to range from 0.7 to 104 µg / kg BW / day with an average of 40 µg / kg BW / day.[i]	1-day spray period with 4-day post-spray urine sampling.	Lavy, *et al.* 1987. Environ. Toxicol. and Chem. 6:209-224.
Exposure to hack-and-squirt workers following special precautions in Arkansas, Oklahoma and Mississippi.	20	not given	Based on urinary excretion, exposure was estimated to range from ND[j] to 60 µg / kg BW / day with an average of 12.2 µg / kg BW / day.[i]	1-day spray period with 4-day post-spray urine sampling.	Lavy, *et al.* 1987. Environ. Toxicol. and Chem. 6:209-224.
Exposure to pilots of aerial application crews in Washington and Oregon - following special precautions.	3[d]	not given	Based on urinary excretion, the authors estimated the average exposure to be 8.54 ± 13.16[e] µg / kg BW / day (no range given).	1-day spray period with 5-day post-spray urine sampling.	Lavy and Mattice, 1984. ACS Symp.238:319-330.
Exposure to mechanics of aerial application crews in Washington and Oregon - following special precautions.	3[d]	not given	Based on urinary excretion, the authors estimated the average exposure to be 3.01 ± 2.69[e] µg / kg BW / day (no range given)	1-day spray period with 5-day post-spray urine sampling.	Lavy and Mattice, 1984. ACS Symp.238:319-330.

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Exposure to batchmen of aerial application crews in Washington and Oregon - following special precautions.	3[d]	not given	Based on urinary excretion, the authors estimated the average exposure to be 14.0±11.7[e] µg / kg BW / day (no range given).	1-day spray period with 5-day post-spray urine sampling.	Lavy and Mattice, 1984. ACS Symp.238:319-330.
Exposure to supervisors of aerial application crews in Washington and Oregon - following special precautions.	3[d]	not given	Based on urinary excretion, the authors estimated the average exposure to be 0.01±0.22 µg / kg BW / day (no range given).	1-day spray period with 5-day post-spray urine sampling.	Lavy and Mattice, 1984. ACS Symp.238:319-330.

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APPENDIX B

TABLE 1. RESULTS OF *IN VITRO* GENOTOXICITY TESTING OF 2,4-D

Test Type	2,4-D Species Tested	Concentration or Dose	Result	Metabolic Activation	Reference
***In Vitro* Mutagenicity Studies - Bacteria**					
Ames (5 strains tested, but not stated)	2,4-D	not stated	negative	with and without liver extract	Ercegovich and Rashid, 1977
Ames (5 strains tested, but not stated)	2,4-D ethyl ester	not stated	positive; stated as "pronounced" mutagenicity	without liver extract	Ercegovich and Rashid, 1977
Ames (5 strains tested, but not stated)	2,4-D butyl ester	not stated	positive; stated as "weak" mutagenic response	without liver extract	Ercegovich and Rashid, 1977
Ames (TA98, TA100, TA1535, TA1538)	not stated	up to 5000 µg / plate	negative	with and without rat S9	Simmon *et al.*, 1977
Ames (TA98, TA100, TA1535, TA1538)	2,4-D acetate	0.1 ml / plate	negative	with and without Aroclor 1254 induced rat liver extract	Anderson and Styles, 1978
Ames (strains not stated)	not stated	not stated	negative	with and without rat S9	Rashid, 1979
Ames (*his* revertants)	not stated	not stated	negative	through host mediation	Styles, 1973
Ames (all strains tested not stated)	2,4-D acid	0 to 1000 µg / plate	negative, but weak positive response at 250 to 750 µg / plate in TA97 with rat S9	with and without rat S9	Kappas *et al.*, 1984
Ames (TA97, TA98, TA100, TA1535, TA1537, TA1538)	not stated, from Serva Feinbiochemica, Germany	0 to 1000 µg / plate	negative, but marginally positive in strain TA97 at 250 to 750 µg / plate with metabolic activation	with and without rat S9	Kappas, 1988
Ames (TA97, TA98, TA100, TA1535, TA1538	not stated	0 to 5000 µg / plate	negative; except for positive in strain TA97, in the presence of metabolic activation at a dose	with and without rat S9	Kappas and Markaki, 1988

Test Type	2,4-D Species Tested	Concentration or Dose	Result	Metabolic Activation	Reference
			of 250 to 750 µg / plate		
Ames (TA1538, TA1978)	not stated	0 to 2000 µg / plate	negative	not stated	Rashid and Mumma, 1986
Ames (TA97, TA98, TA100, TA1535, TA1538)	alanine, aspartic acid, leucine, methionine and tryptophan conjugates	0 to 1000 µg / plate	negative	with rat and woodchuck S9	Rashid *et al.*, 1984
Ames (TA100, TA1535, TA1537, TA1538)	not stated	not stated	negative	with and without rat S9	Waters *et al.*, 1980
Salmonella typhimurium / arabinose-resistant assay system (SV50)	2,4-D acid 2,4-D isooctyl ester	0 to 3333 µg / plate	negative	with and without rat or hamster S9	Soler-Niedziela *et al.*, 1988
Salmonella typhimurium / arabinose-resistant assay system (SV50)	2,4-D n-butyl ester	0 to 10000 µg / plate	negative	with and without rat or hamster S9	Soler-Niedziela *et al.*, 1988
E. coli (WP2)	not stated	not stated	negative; "degradation" products stated to be mutagenic, no other details available	with and without rat S9	Rashid, 1979
E. coli (WP2)	not stated	not stated	negative	with and without rat S9	Waters *et al.*, 1980
E. coli (K12)	not stated	not stated	negative; "degradation" products said to be mutagenic, no other details available	with and without rat S9	Rashid, 1979
E. coli (K12, WP2)	not stated	0 to 2000 µg / plate	negative	not stated	Rashid and Mumma, 1986
E. coli (PQ37)	not stated	0 to 200 µg in 20 µl DMSO	negative (toxic)	with and without rat S9 (Aroclor 1254 induced)	Mersch-Sundermann *et al.*, 1989
***In Vitro* Mutagenicity Studies - Fungi**					
Saccharomyces cerevisiae (D3)	not stated	0.2 ml in 2 ml	negative	with and without rat	Simmon *et al.*, 1977

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Test Type	2,4-D Species Tested	Concentration or Dose	Result	Metabolic Activation	Reference
		phosphate buffer		S9	
Saccharomyces cerevisiae (D3)	not stated	not stated	negative	with and without rat S9	Waters *et al.*, 1980
Aspergillus nidulans	not stated	1 to 10 µg / ml	positive for mitotic crossing over when rat S9 was present	with and without rat S9	Kappas and Markaki, 1988
Aspergillus nidulans	not stated, from Serva Feinbiochemica, Germany	4 to 48 µM	positive for mitotic crossing over at all tested concentrations	with and without rat S9	Kappas, 1988
Aspergillus nidulans	2,4-D acid	up to 10 µg / ml	negative for *meth* suppressor mutations	not stated	Kappas *et al.*, 1984
***In Vitro* Mutagenicity Studies - Mammalian Cells**					
Chinese hamster V79 fibroblasts	not stated	10 to 100 µg / ml for one hour	positive; significant dose-dependent increase in HGPRT locus mutations	none used	Pavlica *et al.*, 1991
***In Vitro* Genotoxicity Studies - Plants**					
Hordeum vulgare	not stated, from E-Merck, Germany	seeds immersed in 2,4-D solution (dose approx. 100 µg / g)	positive; induced mitotic and meiotic chromosomal aberrations (*i.e.*, chromosome clumping, precocious movement, bridges and laggards) and chlorophyll deficiency mutations	none used	Khalatkar and Bhargava, 1985
Crepis capillaris	sodium salt	3 hr. treatment with 0 to 1.0% 2,4-D solutions	positive; induced chromosomal aberrations (chromatid and isochromatid breaks) in G_1 and S cell phases; S-phase was most sensitive	none used	Sidorov *et al.*, 1988
Allium ascalonicum	not stated	2 and 24 hour treatments with 45 and 450 µM 2,4-D solutions	positive; induced chromosomal aberrations (*i.e.*, C-mitosis, bridges, fragmentation, laggards and micronuclei) and reduced mitotic index	none used	Pavlica *et al.*, 1991
***In Vitro* Genotoxicity Studies - Mammalian Cells**					
Chinese hamster ovary cells	not stated	50 to 299 µg / ml	positive; induced sister chromatid exchange at	without rat S9	Galloway *et al.*, 1987

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			doses of 167 to 198 µg / ml		
Chinese hamster ovary cells	not stated	500 to 4200 µg / ml	negative for sister chromatid exchange	with rat S9	Galloway *et al.*, 1987
Chinese hamster ovary cells	not stated	500 to 920 µg / ml	negative for chromosomal aberrations	without rat S9	Galloway *et al.*, 1987
Chinese hamster ovary cells	not stated	1900 to 5000 µg / ml	positive; induced chromosomal aberration at 2400 µg / ml when a 10.5 hour harvest time was used; negative when 19 hour fixation time was used	with rat S9	Galloway *et al.*, 1987
Cell transformation bioassay (human lung W1-38)	2,4-D acetate	0 to 250 µg / ml	negative	with and without rat S9	Styles, 1978
Cell transformation bioassay (baby Syrian hamster kidney cells)	2,4-D acetate	0 to 250 µg / ml	negative	with and without rat S9	Styles, 1978
Human peripheral lymphocytes	2,4-D alkanolamide	human blood treated at G_1 with 0 to 500 ppm	positive; statistically increased (at 250 and 500 ppm) numbers of DNA structural aberrations (mainly of the chromatid type) negative for sister chromatid exchange	none used	El-Zoka and McKenzie, 1980
Human fibroblasts (PM2 DNA)	2,4-D dimethylammonium salt	0 to 100 mmol / l for 45 minutes	positive; induced dose-dependent single strand DNA breaks in apurinic / apyrimidinic PM2 DNA and greatly reduced the colony forming ability	none used	Clausen *et al.*, 1990
Human fibroblasts (PM2 DNA)	2,4-D trimethylammonium salt	0 to 100 mmol / l for 45 minutes	negative; did not induce single strand DNA breaks at apurinic / apyrimidinic sites	none used	Clausen *et al.*, 1990
Human fibroblasts (PM2 DNA)	2,4-D acid	0 to 100 mmol / l for 45 minutes	negative; did not induce single strand DNA breaks at apurinic / apyrimidinic sites	none used	Clausen *et al.*, 1990
Human fibroblasts (cell line GM 5757)	2,4 dimethylammonium salt	0 to 15 mmol / l for one hour	negative; no effect on unscheduled DNA synthesis; dose-dependent decrease in colony forming ability and growth; threshold effects (10 mmol / l) on DNA and protein synthesis	none used	Jacobi and White, 1991
Human fetal lung fibroblasts	not stated	not stated	negative for unscheduled DNA synthesis	with and without	Waters *et al.*, 1980

(continued)

(WI-38 cell line)				metabolic activation	
Other Short-Term Tests					
Rat (Wistar) liver cell endoplasmic recticulum degranulation assay	2,4-D acetate	12 µg / ml incubated for 2 hours	positive; induced a degranulation at an incidence rate of 15.3%	liver preparation contained metabolic activation potential	Lefevre, 1978
Mouse (Swiss) sebaceous gland test	2,4-D acetate	twice daily topical applications of 2.4 mg / mouse for three days	negative; no significant difference in the ratios of sebacious glands to hair follicles between control and treated animals	intact animal	Longstaff, 1978
Mouse (Swiss) skin tetrazolium reduction test	2,4-D acetate	1% solution in benzene	negative	intact mouse skin	Westwood, 1978
DNA-cell-binding assay (Ehrlich ascites cells, *E.coli* cells and *E. coli* DNA)	not stated	20 and 200 µM for 30 or 60 minutes	positive; induced DNA cell binding at 200 µM which was enhanced by the presence of either lysozyme or liver extract; degree of DNA-cell-binding was low compared to many of the other chemicals tested	with and without rat liver extract	Kubinski *et al.*, 1981
Polyamine biosynthesis and cell growth in Chinese hamster ovary cells	not stated	1 mM for 24 hours	positive; significant reduction of protein and DNA biosynthesis as well as ornithine decarboxylase activity (polyamine biosynthesis), effects on cell growth were counteracted by the addition of either 0.1 mM putrescine, spermidine, spermine or polyamines	none used	Rivarola and Balegno, 1991a
Polyamine biosynthesis in Chinese hamster ovary cells	not stated	1 mM for 24 hours	positive; greatly reduced ornithine decarboxylase activity as well as protein and DNA synthesis; addition of spermidine or spermine reversed the 2,4-D effects on protein and DNA synthesis	none used	Rivarola and Balegno, 1991b

TABLE 2. RESULTS OF *IN VIVO* GENOTOXICITY TESTING OF 2,4-D

(continued)

Test Type	2,4-D Species Tested	Dose	Result / Comments	References
Studies Previously Reviewed by CCT (1987)				
Lymphocyte SCE in Occupationally Exposed Humans	2,4-D amine salts and esters	Not determined	No significant effect of occupational exposure to 2,4-D on SCE frequency was observed. No dose-response relationship was apparent between measured 2,4-D concentrations in the urine and SCEs determined in isolated lymphocytes. Subjects who smoked had significantly elevated numbers of SCEs as opposed to the non-smokers.	Linnainmaa (1983a,b) and Linnainmaa and Vainio (1983)
Lymphocyte Chromosomal Aberrations in Exposed Humans	2,4-D acid	Not determined	No differences in the frequency of chromosomal aberrations between workers exposed to 2,4-D and control workers. Smokers had elevated numbers of chromosomal aberrations in both exposed and control groups.	Mustonen *et al.* (1986)
Lymphocyte SCE in Rats	2,4-D acid	100 and 200 mg / kg / day via gavage for one week	No increase in the number of SCEs in the peripheral lymphocytes.	Linnainmaa (1984)
Mouse Bone Marrow Chromosomal Aberrations	not clearly stated	10, 50, 100 and 300 mg / kg	An increase in chromosomal aberrations was observed in the bone marrow cells of animals treated at the two highest dose levels. No increase observed at the 10 and 50 mg / kg dose levels. The results of the higher dose groups are questionable in that the high dose corresponded to the LD_{50}.	Pilinskaya (1974)
Mouse Bone Marrow SCE (C57BL / 6N mice)	2,4-D acid	20 and 40 mg / kg / day in the diet for 8 weeks	No significant elevation of SCE as compared to control animals.	Lamb *et al.* (1981)
Chinese Hamster Bone Marrow SCE	not clearly stated	100 mg / kg / day via gavage for 7 days	No elevation of the number of SCEs in response to 2,4-D treatment was observed.	Linnainmaa (1984)
Mouse Micronucleus Test	not clearly stated	single 100 mg / kg body weight dose via intraperitoneal injection	No effects were observed at the dose utilized.	Jenssen and Renberg (1976)
Drosophila melanogaster sex-linked recessive lethal test	not stated	0, 4.5, and 9.0 mM	Negaive for all of the three broods tested	Vogel and Chandler, 1974
Studies Not Previously Reviewed by CCT (1987)				

(continued)

Test Type	2,4-D Species Tested	Dose	Result / Comments	References
Lymphocyte Chromosomal Aberrations in Exposed Humans	not clearly stated	Not determined	In workers the mean number of chromosomal aberrations (gaps and breaks) increased from off-season to mid-season testing periods. Predominant exposures were to 2,4-D, atrazine, and amitrole although eleven other herbicides were also used. Confounding factors such as smoking were not taken into account, and only 25 metaphases per worker were examined.	Yoder *et al.* (1973)
Lymphocyte SCE and Chromosomal Aberrations in Exposed Humans	not clearly stated	Not determined	The mean number of chromosomal breaks and SCE in the peripheral lymphocytes of 15 Australian soldiers who fought in the Vietnam war were not significantly different from eight controls with no history of herbicide exposure through employment in agriculture or industry.	Mulcahy (1980)
Lymphocyte SCE in Exposed Humans	not clearly stated	Not determined	SCE frequency was studied in 57 herbicide and pesticide sprayers in New Zealand. No increases in SCE were observed in those workers who had protection, while small, but significantly, higher rates of SCE were observed in workers classified as using no protection. Exposures were mainly to 2,4-D and 2,4,5-T, although nearly 30 other chemicals may also have been used by the workers. Confounding factors such as smoking were not taken into consideration.	Crossen *et al.* (1978)
Lymphocyte Chromosomal Aberrations in Exposed Humans	not clearly stated	Not determined	No significant increases in the numbers of chromosomal aberrations were found in ten Swedish workers who had worked with pesticides for 2 to 29 years (average 13). Most common pesticides used were MCPA, mecoprop and 2,4-D. 200 cells per subject were used for the analysis.	Hogstedt and Westerlund (1980)
Chromosome Damage in Exposed Humans	Agent Orange	Not determined	A significantly greater number of chromosome breaks was observed in the lymphocytes from males who had been exposed to Agent Orange in comparison to their wives and children who served as controls. Only 50 cells were examined in each of 10 exposed individuals and the "controls" were not matched for age, sex or smoking status. There was also considerable overlap in the frequency of chromosome damage in the control and exposed groups.	Kaye *et al.* (1985)
Rat Lymphocyte SCE Assay	2,4-D acid	0 to 5 µg / kg via gavage 5 days / week for 2 weeks	No induction of SCEs in rat lymphocytes was observed as a result of treatment with 2,4-D.	Mustonen *et al.* (1989)
Rat Bone Marrow Chromosomal Aberration Assay (male Wistar	2,4-D acid	2 consecutive daily doses, via i.p. injection, of 0, 17.5,	Significantly increased frequency of chromosome breaks and percentage of aberrant cells at the two highest doses; pulverized, ring and dicentric chromosomes were observed when compared to water-based controls.	Adhikari and Grover (1988)

(continued)

Test Type	2,4-D Species Tested	Dose	Result / Comments	References
rat)		35 or 70 mg / kg / day	However, the results for 2,4-D were similar to those reported for the DMSO controls.	
Rat Bone Marrow Chromosomal Aberration Assay (Charles River rat)	not stated	0 to 350 mg / kg via i.p. injection for either 4 or 24 hours	Increased number of chromosome breaks at the 75, 100 and 150 mg / kg dose levels could not be reproduced in 2 additional replicates.	Turkula and Jalal (1987)
Rat Bone Marrow Chromosomal Aberration Assay (male albino rat)	2,4-D butyl ether	1 mg / kg via gavage six times / week for 6.5 months	Weak cytogenetic effects reported with an increased coefficient of mitotic phases and chromosomal damage (chromatid bridges and adhesions) at 2.5 months and damage to the mitotic apparatus at 6.5 months. This study was described in a Russian journal with no raw data or experimental details available for evaluation.	Konstantinova and Shevelo (1984)
Mouse Micronucleus Assay (CD-1 mice)	2,4-D acid	1 / 32 to 1 / 4 of the dermal LD_{50}	No induction of micronuclei in bone marrow.	Schop *et al.* (1990)
Mouse Micronucleus Assay	2,4-D acid	not stated	Test was considered to be inadequate for evaluation.	EPA Gene-Tox Program I (in Mavournin *et al.*, 1990)
Mouse Hair Follicle Nuclear Aberration Assay (male CD-1 mice)	2,4-D acid	1 / 32 to 1 / 4 of the dermal LD_{50}	Induction of nuclear aberration in the hair follicles was observed at the highest dose tested (1 / 4 of the LD_{50}). This particular test has not been validated for the detection of carcinogens, and certain non-carcinogens (*e.g.*, DMSO) also induce positive results in this and other nuclear anomaly assays.	Schop *et al.* (1990)
Mouse Testicular DNA Synthesis Assay	2,4-D acid	single 200 mg / kg oral dose	Induced a significant ($p<0.05$) 29% inhibition of testicular DNA synthesis as measured by thymidine uptake. This test is considered to be unreliable and has been discontinued from use in the screening of potential chemical carcinogens.	Seiler (1979)
Drosophila melanogaster sex-linked recessive lethal test	not stated	0 and 1000 ppm	Negative for complete and partial chromosome loss.	Woodruff *et al.*, 1983
Drosophila melanogaster sex-linked recessive lethal test	2,4-D acid	0, 1000, and 10000 ppm via injection or diet	Negative for all broods tested.	Zimmering *et al.*, 1985

(continued)

APPENDIX C

Appendix C contains a compilation of the studies on the acute toxicity of various chemical forms of 2,4-D. Cases of acute and subchronic health effects have also been reported in individuals who have experienced poisoning or who were exposed occupationally to toxic doses. Many effects are well-known and have been reported extensively in the literature (Gleason *et al.*, 1963; Bleiberg *et al.*, 1964; Bashirov, 1969; Balo-Banga *et al.*, 1975; Torrington, 1983; McMillin and Samples, 1985; Durakovic and Durakovic, 1987; Smith and Lewis, 1987; Kancir *et al.*, 1988; Ellenhorn and Barceloux, 1988; Flanagan *et al.*, 1990).

ACUTE TOXICITY OF 2,4-D IN EXPERIMENTAL ANIMALS

Strain (number per group)	Chemical Species	Route	LD_{50} (mg/kg/day)	Reference
rat (Wistar, male) (27 animals total)	2,4-D acid	oral	980±83	Ylitalo *et al.*, 1990 (Gen Pharmac 21(5):811)
rat (male) (5/group)	2,4-D acid (95% active)	oral	639 (acid equiv[a] 607)	ITF, 1992 (3.1.2.1); Gorzinski *et al.*, 1987
rat (female) (5/group)	2,4-D acid (95% active)	oral	764 (acid equiv 726)	ITF, 1992 (3.1.2.1); Gorzinski *et al.*, 1987
rat (combined sexes)	2,4-D acid	oral	699	ITF, 1992 (3.1.2.1)
rat (Sprague-Dawley, female) (9/group)	2,4-D acid	oral	920	Kitchen and Brown, 1988 (Toxicol Environ Chem 16:165)

Strain (number per group)	Chemical Species	Route	LD_{50} (mg / kg / day)	Reference
rat (5 / sex / group)	2,4-D, isobutyl ester	oral	618	ITF, 1992 (3.1.2.2)
rat (male) (5 / group)	2,4-D, isobutyl ester	oral	700 (acid equiv 536)	EPA, 1989 (Pesticide Fact Sheet); Gorzinski *et al.*, 1987
rat (female) (5 / group)	2,4-D, isobutyl ester	oral	553 (acid equiv 424)	EPA, 1989 (Pesticide Fact Sheet); Gorzinski *et al.*, 1987
rat (male) (5 / group)	2,4-D, butoxyethanol ester	oral	887 (acid equiv 564)	Gorzinski *et al.*, 1987
rat (female) (5 / group)	2,4-D, butoxyethanol ester	oral	831 (acid equiv 565)	Gorzinski *et al.*, 1987
rat (5 / sex / group)	2,4-D, butoxyethanol ester	oral	850	ITF, 1992 (3.1.2.3)
rat	2,4-D, butoxyethyl ester	oral	866	EPA, 1989 (Pesticide Fact Sheet)
rat (male) (5 / group)	2,4-D, butyl ester	oral	732 (acid equiv 575)	Gorzinski *et al.*, 1987
rat (female) (5 / group)	2,4-D, butyl ester	oral	600 (acid equiv 519)	Gorzinski *et al.*, 1987
rat (5 / sex / group)	2,4-D, butyl ester	oral	695	ITF, 1992 (3.1.2.5)
rat (5 / sex / group)	2,4-D, isooctyl ester	oral intubation	896	ITF, 1992 (3.1.2.6)
rat (male) (5 / group)	2,4-D, isooctyl ester	oral	982 (acid equiv 612)	EPA, 1989 (Pesticide Fact Sheet); Gorzinski *et al.*, 1987
rat (female) (5 / group)	2,4-D, isooctyl ester	oral	>720 but <864	EPA, 1989 (Pesticide Fact Sheet); Gorzinski *et al.*, 1987
rat (male)	2,4-D, isopropyl ester	oral	640	EPA, 1989 (Pesticide Fact Sheet)
rat (female)	2,4-D, isopropyl ester	oral	440	EPA, 1989 (Pesticide Fact Sheet)
rat (male) (5 / group)	2,4-D, dimethylamine salt	oral	1090 (acid equiv 619)	Gorzinski *et al.*, 1987
rat (female) (5 / group)	2,4-D, dimethylamine salt	oral	863 (acid equiv 490)	Gorzinski *et al.*, 1987
rat (5 / sex)	2,4-D, dimethylamine salt	oral intubation	949	ITF, 1992 (3.1.2.7)

(continued)

Strain (number per group)	Chemical Species	Route	LD_{50} (mg/kg/day)	Reference
rat (5/sex)	2,4-D, sodium salt	oral	997	ITF, 1992 (3.1.2.4)
rat (male) (5/group)	2,4-D, sodium salt	oral	876 (acid equiv 754)	EPA, 1989 (Pesticide Fact Sheet); Gorzinski *et al.*, 1987
rat (female) (5/group)	2,4-D, sodium salt	oral	975 (acid equiv 840)	EPA, 1989 (Pesticide Fact Sheet); Gorzinski *et al.*, 1987
rat (male)	2,4-D, diethanolamine salt	oral	>2000	EPA, 1989 (Pesticide Fact Sheet)
rat (female)	2,4-D, diethanolamine salt	oral	1605	EPA, 1989 (Pesticide Fact Sheet)
mouse	2,4-D acid	oral	368 (312-434)	Rowe and Hymas, 1954 (cited in: Gehring and Betso, 1978 (Ecol Bull 27:122-133))
dogs	2,4-D acid	oral	100 (25-250)	Rowe and Hymas, 1954 and Seaburg, 1963 (cited in: Gehring and Betso, 1978 (Ecol Bull 27:122-133))
guinea pigs	2,4-D acid	oral	469 (397-553)	Rowe and Hymas, 1954 (cited in: Gehring and Betso, 1978 (Ecol Bull 27:122-133))
chickens	2,4-D acid	oral	541 (358-817)	Rowe and Hymas, 1954 (cited in: Gehring and Betso, 1978 (Ecol Bull 27:122-133))
rat	2,4-D, sodium salt	dermal	>2000	EPA, 1989 (Pesticide Fact Sheet)
rabbit	2,4-D	dermal	1400	Lehman, 1952 (cited in: Gehring and Betso, 1978 (Ecol Bull 27:122-133))
rabbit (5/sex)	2,4-D, isobutyl ester	dermal	>2000	ITF, 1992 (3.1.3.2)
rabbit (5/sex)	2,4-D, butoxy ethanol ester	dermal	>2000	ITF, 1992 (3.1.3.3)
rabbit (5/sex)	2,4-D, sodium salt	dermal	>2000	ITF, 1992 (3.1.3.4)
rabbit (5/sex)	2,4-D, butyl ester	dermal	>2000	ITF, 1992 (3.1.3.5)
rabbit (5/sex)	2,4-D, isooctyl ester	dermal	>2000	ITF, 1992 (3.1.3.6)
rabbit (5/sex)	2,4-D, dimethylamine salt	dermal	2244	ITF, 1992 (3.1.3.7)

(continued)

Strain (number per group)	Chemical Species	Route	LD_{50} (mg/kg/day)	Reference
rabbit	2,4-D, diethanolamine	dermal	>2000	EPA, 1989 (Pesticide Fact Sheet)
rabbit	2,4-D, isopropyl ester	dermal	>2000	EPA, 1989 (Pesticide Fact Sheet)
rabbit (female)	2,4-D, butoxyethyl ester	dermal	>2000	EPA, 1989 (Pesticide Fact Sheet)
rabbit (male)	2,4-D, butoxyethyl ester	dermal	1829	EPA, 1989 (Pesticide Fact Sheet)
rat	2,4-D acid	inhalation	1.79 mg/l	EPA, 1989 (Pesticide Fact Sheet)
rat	2,4-D, diethanolamine salt	inhalation	>3.8 mg/l	EPA, 1989 (Pesticide Fact Sheet)
rat	2,4-D, butoxyethyl ester	inhalation	>4.6 mg/l	EPA, 1989 (Pesticide Fact Sheet)
rat	2,4-D, isopropyl ester	inhalation	>4.97 mg/l	EPA, 1989 (Pesticide Fact Sheet)

[a]2,4-D acid equivalents

(continued)

TABLE 1. SUMMARY TABLE OF HUMAN EXPOSURE TO 2,4-D IN WHICH PROTECTIVE CLOTHING WAS WORN

(from table in Appendix A)

Exposure Group	Number of Subjects	Range of Measured Internal Dose (µg / kg BW / day)	Average Estimated Internal Dose (µg / kg BW / day)	Reference
HOME AND GARDEN USERS				
Home and garden use of 2,4-D: granular liquid Arithmetic mean	 11 11 	 ND[k] to 1.54 ND[a] to 0.9 	 0.14[l] 0.13[m] 0.14	Harris *et al.*, 1992
BYSTANDERS				
Bystanders to 2,4-D use following home and garden use: granular liquid	 11 10	 all subjects ND[a] all subjects ND[a]	 ND[a] ND[a]	Harris *et al.*, 1992
Bystanders to 2,4-D use following aerial application	6[d]	not given	0.09±0.23[e]	Lavy and Mattice, 1984
FARM WORKERS				
Farm workers involved in spraying fields from a ground rig[f]	8	2.14 to 15[g] µg / kg BW / spray operation[h]	5.78	Grover *et al.*, 1986
COMMERCIAL APPLICATORS				
Lawn care specialists	8	not given	0.35±0.17[i]	Yeary, 1986

Exposure Group	Number of Subjects	Range of Measured Internal Dose (µg / kg BW / day)	Average Estimated Internal Dose (µg / kg BW / day)	Reference
	12		1.38±0.5[i]	
	13		3.2±1.0[i]	
	12		6.3±1.8[i]	
	12		2.5±1.5[i]	
Arithmetic mean			2.75	
FORESTRY WORKERS				
A) Aerial Application Crews				
Mixers / Loaders / Batchmen	2	0.53 to 4.52	2.52	Frank *et al.*, 1985
	1	--	11.3	
	3[d]	not given	14.0±11.7[e]	Lavy and Mattice, 1984
Arithmetic mean			9.27	
Pilots	3[d]	not given	8.54±13.16[e]	Lavy and Mattice, 1984
Mixer and balloon men	1	--[j]	4.95	Frank *et al.*, 1985
Balloon men	1	--	3.91	Frank *et al.*, 1985
Supervisors	1	--	0.22	Frank *et al.*, 1985
	3[d]	not given	0.01±0.22[e]	Lavy and Mattice, 1984
Arithmetic mean			0.12	
Mechanics	3[d]	not given	3.01±2.69[e]	Lavy and Mattice, 1984

B) Ground Crews				
Backpack workers	20	30 to 244	98[k]	Lavy *et al.*, 1987
Injection bar workers	20	ND[l] to 12.1	4.3[k]	Lavy *et al.*, 1987
Hypohatchet workers	20	0.7 to 104	40[k]	Lavy *et al.*, 1987
Hack-and-squirt workers	20	ND[l] to 60	12.2[k]	Lavy *et al.*, 1987

TABLE 2. EPIDEMIOLOGY PERTINENT TO 2,4-D AND CANCER

Date	First Author	Study Type	Exposure	Reported Findings	Comments
2,4-D					
1981 Sep 12	Olsson H	case-control, letter	2,4-D and other phenoxy herbicides	Five out of 123 cases of NHL had cutaneous lesions, and four of these were sprayers.	Case series with no control group.
1986 Sep 5	Hoar SK	case-control	2,4-D	Associated with NHL in farmers in Kansas.	Findings based on seven cases peculiarly reporting use of herbicide 21 or more days per year.
1990	Brown LM	case-control	2,4-D	No significant association between phenoxy acid herbicides and leukemia in Iowa and Minnesota farmers.	Exposure information based on recall; information bias possible.
1990 Jan	Weisenburger D	case-control, abstract	2,4-D	Significantly associated with NHL in Kansas and Nebraska when data from two studies combined.	Exposure information based on patients' recall; finding driven by peculiar reporting of use of phenoxy acid herbicides 20 or more days per year.
1990 Sep	Zahm SH	case-control	2,4-D	Trend in data suggests an association between 2,4-D and NHL in Nebraska.	Exposure information based on recall of patients and next of kin; trend test driven by three cases with use of 2,4-D reported as being 20 or more days per year.
1990 Mar	Hogan DJ	case-control	2,4-D	Significant risk factor for squamous cell carcinoma of the skin in Saskatchewan.	Subjective exposure information based on patients' recall.
1990	Weisenburger DD	case-control	2,4-D	Statistically insignificantly associated with NHL in Nebraska.	Exposure based on recall; numbers of cases small and finding highly dependent on lack of misclassification of exposure.
1991 Sep 4	Hayes HM	case-control	2,4-D	Increased risk of canine malignant lymphoma at households using 2,4-D on lawn.	No exposure information directly relevant to phenoxy acid herbicide usage.

Date	First Author	Study Type	Exposure	Reported Findings	Comments
1992 May 1	Cantor KP	case-control	2,4-D	Small increased risks that did not increase with latency or lack of protective equipment for NHL among farmers in Iowa and Minnesota.	Pattern of findings within the study not suggestive of a link with phenoxy acid herbicides.
1974	Axelson O	cohort (SMR[a])	2,4-D, 2,4,5-T and amitrol	No significantly increased tumor incidence in Swedish railroad workers potentially exposed to phenoxy acid herbicides.	Limited power in the phenoxy acid worker group.
1976 Sep	Barthel E	cohort (SMR), abstract	2,4-D and other pesticides including MCPA, DDT, HCH, Toxaphen, Parathion, DNOC, copper-containing fungicides, arsenic	Lung cancer in German agricultural pesticide workers 2 times higher than in age-specific general population.	Cigarette smoking not considered.
1980	Axelson O	cohort (SMR)	2,4-D, 2,4,5-T and amitrol	Slightly increased tumor mortality among Swedish railroad workers, but no type of tumor predominated.	Only 816 person-years of observation for phenoxy acid group; subjective exposure assessment.
1980	Hogstedt C	cohort (SMR)	2,4-D, 2,4,5-T and DDT	Significantly increased risk of tumors in Swedish forestry workers.	Small numbers of observed cases; subjective exposure information.
1981	Barthel E	cohort (SMR)	2,4-D, MCPA and other pesticides	Significantly increased lung cancer mortality with positive correlation between employment duration and mortality in German agricultural workers.	Exposure information insufficient to address phenoxy acid herbicides.
1982	Riihimaki V	cohort (SMR)	2,4-D and 2,4,5-T	Decrease in overall mortality, no increase in cancer mortality and no deaths from lymphomas or STSs seen in sprayers in Finland.	Relatively small cohort and short follow-up time.
1983 Oct 26	Environmental Health Associates	cohort (SMR)	2,4-D and other agricultural chemicals	No significant mortality excesses among manufacturers and formulators in Richmond.	Small numbers of observed deaths; limited power to detect risk increase.
1985 Aug	Lynge E	cohort (SMR)	2,4-D (possibly), MCPA and small amounts of	STS statistically insignificantly elevated in Danish manufacturers and packagers; no	Exposure information based on records from manufacturers.

Date	First Author	Study Type	Exposure	Reported Findings	Comments
			2,4,5-T	increase in malignant lymphoma.	
1987	Green LM	cohort (SMR), letter	2,4-D and other phenoxy acid herbicides, including 2,4,5-T	No excess cancers in forestry workers.	Subjective exposure information.
1988	Bond GG	cohort (SMR)	2,4-D	Significantly elevated cancer of other and unspecified sites in the entire cohort and all lymphopoietic cancer and cancer of ill-defined sites in the 2,4-D plant in Michigan workers manufacturing, formulating and packaging 2,4-D.	No pattern suggestive of herbicide-related risk.
1990 Nov 5	Bloemen LJ	cohort (SMR), thesis	2,4-D	No indication of causal relationship with any cause of death for 2,4-D manufacturing, formulating and packaging workers in Michigan except significant association with cancer of "other and unspecified sites" in 2,4-D plant.	Long follow-up but limited power.
1991 Mar 5	Vineis P	cohort (SMR)	2,4-D and 2,4,5-T	Two-fold higher incidence of NHL in municipalities where phenoxy acid herbicides identified as soil and water contaminants, but no association with HD or STS.	Ecological exposure assessment.
1992	Hansen ES	cohort (SMR)	2,4-D and other pesticides	STS and chronic lymphatic leukemia significantly increased, and NHL statistically insignificantly increased, in Danish gardeners.	No exposure information relevant to phenoxy acid herbicides.
ACID-CONTAINING CHEMICALS AND CHLORINATED HYDROCARBONS					
1989	Malone KE	case-control	acid-containing chemicals, chlorinated hydrocarbons	Significantly increased risks for chronic lymphatic leukemia.	Crude exposure measures employed.
AGENT ORANGE					
1981 Nov	Greenwald P	case-control	Agent Orange	No association with STS in U.S. Vietnam	Limited power.

Date	First Author	Study Type	Exposure	Reported Findings	Comments
				veterans.	
1987	Kang H	case-control	Agent Orange	Statistically insignificant higher risk for STS when compared to other Vietnam veterans.	Subjective exposure information.
1982 Nov 30	Brown PG	cohort (no data)	Agent Orange	No significant elevations in death rates for Ranch Handers.	Cohort with known significant exposure to phenoxy acid herbicides.
1990	Wolfe WH	cohort (OR[b])	Agent Orange	Significant excess of basal cell carcinomas in Ranch Handers, but not suspected to be chemical related.	Cohort of men likely exposed to large amounts of phenoxy acid herbicides.
1983 Jun 30	USAF School of Aerospace Medicine	cohort (SMR)	Agent Orange	Statistically insignificant decrease in cancer in Ranch Handers.	Cohort of men likely exposed to considerable amounts of phenoxy acid herbicides.
1985 Nov 29	Wolfe WH	cohort (SMR)	Agent Orange	Favorable mortality in Ranch Handers compared to control group and U.S. population.	Study of exposed men with adequate power to detect less than a doubling in risk.
1990 Oct 10	Michalek JE	cohort (SMR)	Agent Orange	No increased mortality of any site among Ranch Handers.	Group of individuals with known exposures to phenoxy acid herbicides.
AGRICULTURAL CHEMICALS					
1983	Hernberg S	case-control	agricultural chemicals	No association with nasal or sinonasal cancer in Denmark, Finland or Sweden.	Exposure information based only on interviews with a percentage of total group; subjective exposure information based on recall.
AGRICULTURE					
1971	Milham S Jr	case-control	agriculture	Significant association with mortality from leukemia and multiple myeloma, but not HD, reticulum cell sarcoma or other lymphomas, seen in farmers.	Exposure information based on death certificates.
1982	Cantor KP	case-control	agriculture	NHL and reticulum cell sarcoma elevated in Wisconsin farmers.	No exposure information directly relevant to phenoxy acid herbicides; magnitude of risk increase, 22%, was unremarkable.

Date	First Author	Study Type	Exposure	Reported Findings	Comments
1985	Pearce NE	case-control	agriculture and forestry	Significant association with malignant lymphoma and multiple myeloma in those under 65 in New Zealand.	Exposure information based on reports to tumor registry.
1985 Aug	Schumacher MC	case-control	agriculture	Significant association with diagnoses of NHL made from 1952 through 1971 in Utah.	Exposure information subjectively determined through occupations reported on death certificates.
1985 Jul / Aug	Blair A	case-control	agriculture	Chronic lymphatic leukemia (but not acute lymphatic leukemia, acute monocytic leukemia or acute unspecified leukemia) significantly elevated among farmers in Nebraska.	No exposure information directly relevant to phenoxy acid herbicides.
1986b	Pearce NE	case-control	agriculture and forestry	Statistically insignificant association with leukemia in New Zealand.	Exposure information based on reports to tumor registry.
1988	Balarajan R	case-control	agriculture and forestry	Significantly increased malignant lymphoma, lymphosarcoma and reticulum cell sarcoma, and HD in agricultural and forestry workers in England and Wales.	Subjective assessment of exposure to herbicides; misclassification bias likely.
1988	Brownson RC	case-control	agriculture	Statistically insignificant elevated risks for NHL and HD in Missouri farmers.	Magnitude of risk increases unremarkable; multiple comparison problems.
1988	Schumacher MC	case-control	agriculture	No association with NHL in North Carolina.	Exposure information gathered from death certificates.
1989	Reif J	case-control	agriculture	In New Zealand: significant associations with malignant melanoma, lip cancer, rectal cancer, prostate cancer, brain cancer and lymphatic / hematopoietic cancer; statistically insignificant associations with leukemia and NHL; significant negative associations with laryngeal, lung and testicular cancer.	Magnitude of risk increases small with statistical significance driven by large number of subjects studied overall.
1989	Brownson RC	case-control	agriculture	Significant elevations in lip cancer, prostate cancer, NHL, and lymphatic and hematopoietic cancer, but significant decrease in lung cancer in Missouri	Subjective exposure assessment; multiple comparisons that could give rise to false positives.

Date	First Author	Study Type	Exposure	Reported Findings	Comments
				farmers.	
1981 Mar	Burmeister LF	cohort (PMR[c])	agriculture	Significantly elevated lip cancer, stomach cancer, leukemia, lymphatic cancer, multiple myeloma and prostate cancer, but significantly lowered smoking-related cancers, in Iowa farmers.	Risk increases of 10 to 50 percent unremarkable; no exposure information directly relevant to phenoxy acid herbicides.
1982 Jun 26	Milham S Jr	cohort (PMR)	agriculture	Significantly elevated risk of STS in Washington farmers.	Exposure based on death certificate reporting.
1984	Stubbs HA	cohort (PMR)	agriculture	Lymphatic tissue cancer, cervical cancer, stomach cancer, rectal cancer, esophageal cancer and uterine cancer elevated in California farmers.	No exposure data.
1985	Delzell E	cohort (PMR)	agriculture	Increased risks of melanoma and other skin cancer, brain cancer, leukemia and prostate cancer, but no evidence for increased risks of NHL or multiple myeloma, among North Carolina farmers.	No exposure information relevant to phenoxy acid herbicides.
1980 Aug	Cantor KP	cohort (rates)	agriculture and food packing	"Farmer" appeared on NHL death certificates more often in counties with industrial concentration in food packing.	No exposure information relevant to phenoxy acid herbicides.
1968	Fasal E	cohort (SMR)	agriculture	In California farmers, cancer mortality decreased except for leukemia and lymphomas (lymphosarcoma, reticulosarcoma and HD were decreased, though).	No exposure information directly relevant to phenoxy acid herbicides.
1984 Mar	Buesching DP	cohort (SMR), letter	agriculture	Significantly increased risks for NHL and prostate cancer in Illinois farmers.	Small numbers of observed deaths; statistics driven by method of estimating person-years at risk; no exposure information.
1987 Jul / Aug	Stark AD	cohort (SMR)	agriculture	Fewer than expected deaths among farmers for each major category except accidents in New York.	Large study with considerable power.

Date	First Author	Study Type	Exposure	Reported Findings	Comments
1990 May / Jun	Stark AD	cohort (SMR)	agriculture	Lip, melanoma of the skin, prostate, multiple myeloma cancers statistically insignificantly higher than expected, but all other cancers, including NHL, lower than expected in New York farmers.	Large study with considerable power.
HERBICIDES					
1982	Burmeister LF	case-control	herbicides and agriculture	Significantly elevated leukemia mortality which increased with numbers of acres treated with herbicides in Iowa farmers.	Subjective exposure information; magnitude of risk increases unremarkable.
1983	Burmeister LF	case-control	herbicides and agriculture	Iowa farmers had significantly elevated mortality for multiple myeloma, NHL, prostate cancer and stomach cancer. Multiple myeloma and NHL associated with herbicide use.	No exposure information directly relevant to phenoxy acid herbicides.
1989	Franceschi S	case-control	herbicides and agriculture	Did not affect the risk of NHL in Italy.	Subjective exposure information.
1989	La Vecchia C	case-control	herbicides	Significant trend for duration of exposure to herbicide seen for lymphomas in Milan.	Subjective exposure information dependent on patients' recall.
1992 Jan 15	Holly EA	case-control	herbicides, pesticides and fertilizers	Significantly increased risk of Ewing's bone sarcoma in children in San Francisco following paternal exposure.	Questionable biological plausibility.
1989	Bender AP	cohort (SMR)	herbicides, diesel fuels and exhausts, asphalts and tars, gasoline, polynuclear aromatic hydrocarbons, benzene, lead	Mortality in Minnesota highway maintenance workers significantly elevated for leukemia in those with 30-39 years of work and urologic cancer in those with 40-49 year latency.	Specific exposure to phenoxy acid herbicides not studied.
1990 Apr 4	Wigle DT	cohort (SMR)	herbicides	Although no overall increase in NHL risk, significant dose-response relationship between NHL in Saskatchewan farmers and acres sprayed.	Overall deficit of NHL and possible confounding by fuel and oil.

Date	First Author	Study Type	Exposure	Reported Findings	Comments
1990	Thomas TL	cohort (SMR)	herbicides, riot control substances, and burning agents	Two deaths from leukemia (0.5 expected), two deaths from brain cancer (0.4 expected), two HD cases (0.7 expected) and one hairy cell leukemia in Vietnam Army Chemical Corps veterans.	Small cohort of men likely exposed to phenoxy acid herbicides, and possibly confounding substances.
PESTICIDES					
1983 Jul	Blair A	cohort (SMR)	pesticides	In Florida pesticide applicators, leukemia significantly elevated, brain cancers statistically insignificantly elevated and lung cancer significantly elevated, but only in those who had been licensed for 20 or more years.	Small numbers of observed cases; no information on specific exposures; magnitude of increases unremarkable.
1988 May	MacMahon B	cohort (SMR)	pesticides	Significantly elevated standardized mortality ratio for lung cancer in pesticide applicators.	Smoking could not be evaluated.
1990 Sep / Oct	Cantor KP	cohort (SMR)	pesticides	Leukemia elevated, but NHL decreased, among aerial pesticide applicators.	Subjective assessment of exposure.
PHENOXIES					
no date	Smith AH	case-control	phenoxy acids	No association with STS in New Zealand.	Exposure information based on interviews.
1978 Oct	Hardell L	case-control	phenoxy acids and chlorophenols	Six-fold increased risk for STS in Sweden.	Subjective exposure information based on patients' recall.
1979 Jan 6	Hardell L	case-control, letter	phenoxy acids and chlorophenols	Fourteen of 17 male lymphoma patients in Sweden reported a current occupation which could have exposure and 11 reported exposure.	Case series with no control group.
1979 Jun	Hardell L	case-control	phenoxy acids and chlorophenols	Six-fold increased risk for soft-tissue sarcoma in Sweden.	Subjective exposure information based on patients' recall.
1981	Hardell L	case-control	phenoxy acids and chlorophenols	Elevated risks for STS and malignant lymphoma in Sweden.	Subjective exposure information based on patients' recall.

Date	First Author	Study Type	Exposure	Reported Findings	Comments
1981	Hardell L	case-control	phenoxy acids, chlorophenols and organic solvents	Associated with malignant lymphoma in Sweden.	Subjective exposure assessment based on patients' recall.
1981	Eriksson M	case-control	phenoxy acids other than 2,4,5-T	Increased risk of STS in Sweden.	Subjective exposure information.
1982	Hardell L	case-control	phenoxy acids	Doubled but statistically insignificant risk for nasal and nasopharyngeal cancer in Sweden.	Subjective exposure information.
1984 Nov	Smith AH	case-control	phenoxy herbicides including 2,4,5-T	Not associated with STS in New Zealand.	Exposure information based on questionnaire.
1986	Vineis P	case-control	phenoxy herbicides	Statistically insignificant increased risk of STSs in female Italian rice weeders.	Subjective assessment of exposure to pesticides.
1986a	Pearce NE	case-control	phenoxy herbicides	Not associated with NHL in New Zealand.	Exposure information based on questionnaire.
1987	Pearce NE	case-control	phenoxy herbicides	No association with NHL in New Zealand.	Data support oncogenic virus hypothesis for malignant lymphoma.
1987 May	Woods JS	case-control	phenoxy herbicides and other chemicals	Small significant increased risk of NHL in Washington.	Exposure information gathered through questionnaire; trends in study do not suggest association with any specific class of pesticide.
1989	Persson B	case-control	phenoxy acids	Associated with significant increased risk of NHL.	Exposure information based on self-administered mailed questionnaire.
1989	Woods JS	case-control	phenoxy herbicides	Not independently associated with NHL in Washington.	Exposure information based on questionnaire.
1989	Pearce N	case-control, letter with re-analyzed data	phenoxy herbicides	Duration and frequency of use not associated with NHL in New Zealand.	Letter responding to hypothesis that Swedish sprayers receive higher absorbed doses because the spraying season is compacted.
1990 Mar 21	Eriksson M	case-control	phenoxy acids other than 2,4,5-T	No increased risk of STS associated with phenoxy acid herbicides in Sweden.	Subjective exposure information.

Date	First Author	Study Type	Exposure	Reported Findings	Comments
1992	Smith JG	case-control	phenoxy acid herbicides	No association with STS or malignant lymphoma in Australia	Exposure estimates based on questionnaire.
1984 Jul 7	Gallagher RP	cohort (PMR)	phenoxy herbicides	Associated with insignificantly increased STS and NHL in British Columbia farmers and gardeners / nursery workers.	Subjective exposure information regarding phenoxy acid herbicides.
1986 Feb	Wiklund K	cohort (RR[d])	phenoxy acid herbicides	Not associated with STS in Swedish agriculture and forestry workers.	Large study of likely exposed men with considerable power.
1987	Wiklund K	cohort (RR)	phenoxy acid herbicides	Not associated with STS in Swedish agricultural and forestry workers.	Large study of workers with likely exposure to phenoxy acid herbicides; considerable power.
1988a	Wiklund K	cohort (RR)	phenoxy acid herbicides	Significant association with HD, with time-related rising trend in relative risk, in fur farming and silviculture (forestry) workers in Sweden.	Trend and data not consistent with phenoxy herbicides as cause.
1979	Westerlund B	cohort (SMR)	phenoxy acids and DDT	"Tendency toward" increased tumor incidence and tumor mortality in timber workers in Sweden.	Subjective and speculative exposure assessment.
1983	Riihimaki V	cohort (SMR)	phenoxy acids	Cancer morbidity and mortality unremarkable and no cases of STS or lymphoma seen in applicators in Finland.	Small cohort and limited power.
1988b	Wiklund K	cohort (SMR)	phenoxy acid herbicides	Not associated with STS in Swedish pesticide applicators.	Large study of men likely exposed to phenoxy acid herbicides with considerable power.
1989	Wiklund K	cohort (SMR)	phenoxy acid herbicides	Significant association with HD in Swedish pesticide applicators born after 1934, but not with NHL or STS.	Large cohort of men with likely exposure to phenoxy acid herbicides; study has considerable power to detect risk increases.
1991	Saracci R	cohort (SMR)	phenoxy herbicides and chlorophenols	No exess in lymphomas, but a doubled, though statistically insignificant, risk of STS among international production workers and, predominantly, sprayers.	Large group of workers with known and significant exposure to multiple phenoxy herbicides and chlorophenols.

Date	First Author	Study Type	Exposure	Reported Findings	Comments
1991	Coggon D	cohort (SMR)	phenoxy herbicides	Mortality from NHL, but not HD or STS, statistically insignificantly increased in international cohort of manufacturing workers.	Large group of workers with known and significant exposure to phenoxy acid herbicides.
1991	Green LM	cohort (SMR)	phenoxy acids and other herbicides	No deaths from STS and NHL in forestry workers.	Subjective exposure assessment.
VIETNAM					
1986 Dec	Kang HK	case-control	Vietnam	No significant association with STSs.	Subjective exposure information.
1989 Jul	Irey NS	case-control	Vietnam	No significant differences in demography, anatomy or pathology between veterans with and without Vietnam service.	No exposure information directly relevant to phenoxy acid herbicides.
1990 Sep	Centers for Disease Control	case-control	Vietnam	Increased risk of NHL, but no increased risk of STS, HD, nasal or nasopharyngeal cancer or liver cancer in U.S. Vietnam veterans.	Risk increase not associated with exposure to phenoxy acid herbicides.
1991 Jul	Dalager NA	case-control	Vietnam	No increased risk of NHL among U.S. Vietnam veterans.	Exposure to phenoxy acid herbicides could not be estimated.
1991	Clapp RW	cohort (OR)	Vietnam	STS incidence significantly elevated in Massachusetts Vietnam veterans.	No exposure information relevant to phenoxy acid herbicides.
1991 Jun	O'Brien TR	cohort (SMR)	Vietnam	Statistically insignificant increased risk of NHL in Vietnam veterans.	Subjective exposure information with respect to phenoxy acid herbicides.
1991	Thomas TL	cohort (SMR)	Vietnam	Significantly elevated mortality from cancer of pancreas and uterine corpus in female veterans.	No information with respect to potential exposure to phenoxy acid herbicides.

[a] standardized mortality or morbidity ratio
[b] odds ratio
[c] proportional mortality or morbidity ratio
[d] relative risk

TABLE 3. DATA FROM MOST RECENT PRINCIPAL CANCER COHORT FOLLOW-UPS

Cohort	Years After First Exposure	NHL (Observed/ Expected)	HD (Observed/ Expected)	STS (Observed/ Expected)	Other Cancers (Observed/ Expected)	Reference
1658 German pesticide applicators	6-17	5/? (lymphatic system)		1/? (soft tissues)	5/3.0 (lung) as expected (all other)	Barthel, 1981
	18-30				45/24.5 (lung) as expected (all other)	
3827 Florida pesticide applicators	10+	as expected (lymphomas)		as expected	84/75.1	Blair *et al*., 1983
878 Michigan 2,4-D producers, formulators and packagers	3+	2/1.0	1/0.4	0/?	25/29.5	Bloemen, 1990
	15+	0/0.7	1/0.2	0/?	21/23.4	
1535 employees of U.S. manufacturer of agricultural chemicals, pesticides and fertilizers	11+ for 64.3%	0/0.49	0/0.37	?	20/20.59	Environmental Health Associates, 1983
British Columbia farmers	?	89 (PMR[a] for lymphosarcoma and reticulosarcoma) 120 (PMR for other NHL)	94 (PMR)	117 (PMR)	?	Gallagher and Threlfall, 1984
British Columbia gardeners and nursery workers	?	218 (PMR for lymphosarcoma and reticulosarcoma)	93 (PMR)	139 (PMR)	?	

Cohort	Years After First Exposure	NHL (Observed/ Expected)	HD (Observed/ Expected)	STS (Observed/ Expected)	Other Cancers (Observed/ Expected)	Reference
		196 (PMR for other NHL)				
4015 Danish gardeners	10+	8/0.04	?	3/0.0066	206/207.66	Hansen *et al*., 1992
1261 U.S. Air Force Ranch Hands	16-25	0/? (lymphomas)		1/?	12/17.0 (includes STS)	Michalek *et al*., 1990
Washington State white males exposed to phenoxy herbicides and chlorophenols	?	98 (PMR for lymphosarcoma and reticulosarcoma) 105 (PMR for other NHL)	102 (PMR)	120 (PMR)	?	Milham (1982)
1926 Finnish 2,4-D and 2,4,5-T sprayers	10+	0/0.8		0/0.1	20/23.4	Riihimaki *et al*., 1982
18,910 international producers and sprayers of phenoxy herbicides and chlorophenols	<10	1/2.67	below expected	0/0.50	99/91.36 (includes HD)	Saracci *et al*., 1991
	10+	10/9.00		4/1.42	401/403.86 (includes HD)	
954 U.S. Army Chemical Corps Vietnam veterans	16-22	0/? (mortality) 0/0.4 (morbidity)	0/? (mortality) 2/0.2 (morbidity)	0/? (mortality) 0/? (morbidity)	6/6.6 (mortality)	Thomas and Kang, 1990
69,513 Saskatchewan farmers	?	103/112.1	?	?	3095/3841.1	Wigle *et al*., 1990
254,417 Swedish men employed in land/animal husbandry	1+	670/? (0.97 RR[b])	242/? (1.02 RR)	253/? (0.9 RR)	?	Wiklund and Holm, 1986; Wiklund *et al*., 1988a
	13+	0.95 (RR)	1.17 (RR)	0.9 (RR)	?	
11,626 Swedish men	1+	22/? (0.71 RR)	10/? (0.99 RR)	10/? (0.8 RR)	?	

Cohort	Years After First Exposure	NHL (Observed/ Expected)	HD (Observed/ Expected)	STS (Observed/ Expected)	Other Cancers (Observed/ Expected)	Reference
employed in horticulture						
	13+	0.63 (RR)	1.07 (RR)	?	?	
14,126 Swedish men employed in other agricultural occupations	1+	37/? (1.11 RR)	22/? (1.74 RR)	11/? (0.8 RR)	?	
	13+	0.90 (RR)	3.53 (RR)	?	?	
7215 Swedish men employed in silviculture	1+	10/? (0.66 RR)	15/? (2.26 RR)	5/? (0.8 RR)	?	
	13+	0.57 (RR)	3.85 (RR)	?	?	
61,153 Swedish men employed in timber cutting	1+	111/? (0.87 RR)	61/? (1.05 RR)	49/? (1.0 RR)	?	
	13+	0.93 (RR)	0.84 (RR)	0.9 (RR)	?	
6083 Swedish men employed in other forestry occupations	1+	11/? (0.81 RR)	5/? (0.81 RR)	3/? (0.6 RR)	?	
	13+	0.93 (RR)	?	?	?	
20,245 Swedish pesticide applicators	<10	10/11.02	7/6.62	3/3.84	?	Wiklund *et al*., 1989
	10+	17/14.29	8/3.64	4/3.85	?	

[a] proportional mortality or morbidity ratio
[b] relative risk

TABLE 4. SUMMARY OF SUBCHRONIC TOXICITY STUDIES ON 2,4-D

Strain (number per group)	Chemical species	Route	Dose (mg/kg/day) (* denotes acid equivalents)	Duration of Exposure	NOEL/NOAEL[a] (mg/kg/day)	Reference
Fischer F344 rats (15/sex)	2,4-D (100% pure)	diet	0, 15, 60, 100, 150	13 weeks	15 (females only)	Gorzinski *et al.*, 1981a
Fischer F344 rats (15/sex)	2,4-D (97.5% pure)	diet	0, 15, 60, 100, 150	13 weeks	15 (females only)	Gorzinski *et al.*, 1981b
Fischer F344 rats (20/sex)	2,4-D (97.5% pure)	diet	0, 1, 5, 15, 45	13 weeks	1 (males only)	ITF, 1983a
Fischer F344 rats (10/sex)	2,4-D (96.1% pure)	diet	0, 1, 15, 100, 300	13 weeks	15	ITF, 1991a
Fischer F344 rats (10/sex)	2,4-D ethylhexyl ester	diet	0, 1, 15, 100, 300 *	13 weeks	15	ITF, 1991b
Fischer F344 rats (10/sex)	2,4-D dimethylamine salt	diet	0, 1, 15, 100, 300 *	13 weeks	15	ITF, 1991c
Fischer F344 rats (10/sex)	2,4-D butoxyethyl ester	diet	0, 1, 15, 100, 300 *	13 weeks	15	Dow, 1991a
Fischer F344 rats (10/sex)	2,4-D triisopropanolamine salt	diet	0, 1, 15, 100, 300 *	13 weeks	15	Dow, 1991b
Fischer F344 rats (10/sex)	2,4-D isopropylamine salt	diet	0, 1, 15, 100, 300 *	13 weeks	15	Dow, 1991c
male Wistar rats (8/group)	2,4-D sodium salt	intraperitoneal	0, 100, 150	every other day for 12 weeks	--	Lukowicz-Ratajczak and Krechniak, 1988
B6C3F1 mice (10/sex)	2,4-D acid	diet	0, 5, 15, 45, 90	13 weeks	--	ITF, 1983b
B6C3F1 mice (10/sex)	2,4-D acid	diet	0, 1, 15, 100, 300	13 weeks	15	ITF, 1991d
ICR male mice (40-50/group)	2,4-D acid	oral intubation	50, 100, 200	14 days	--	Kuntz *et al.*, 1990
C57Bl/6 male mice (3/group)	2,4-D acid	diet	100	4 days	--	Lundgren *et al.*, 1987
Beagle dog (5/sex)	2,4-D acid	oral gavage in gelatin capsules	0, 0.3, 1, 3, 10	13 weeks	1	ITF, 1990
rabbits (1/sex)	2,4-D acid	dermal	10, 30, 100, 300, 1000	6hr/day, 21 days	--	ITF, 1992 (3.3.3.1)
rabbits (5/sex)	2,4-D acid	dermal	0, 10, 100, 1000	6hr/day, 21 days	1000	ITF, 1992 (3.3.3.4)
rabbits (1/sex)	2,4-D dimethylamine salt	dermal	10, 30, 100, 300, 1000 *	6hr/day, 21 days	--	ITF, 1992 (3.3.3.2)

Strain (number per group)	Chemical species	Route	Dose (mg/kg/day) (* denotes acid equivalents)	Duration of Exposure	NOEL/NOAEL[a] (mg/kg/day)	Reference
rabbits (5/sex)	2,4-D dimethylamine salt	dermal	0, 10, 100, 300 *	6hr/day, 21 days	10	ITF, 1992 (3.3.3.5)
rabbits (1/sex)	2,4-D 2-ethylhexyl ester	dermal	10, 30, 100, 300, 1000 *	6hr/day, 21 days	--	ITF, 1992 (3.3.3.3)
rabbits (5/sex)	2,4-D 2-ethylhexyl ester	dermal	0, 10, 100, 1000 *	6hr/day, 21 days	10	ITF, 1992 (3.3.3.6)

[a]no-observable-adverse-effect level

TABLE 5. DOSE-RESPONSE SUMMARY - KIDNEY EFFECTS IN RATS

Dose Level	Sex	Effects	Incidence	Reference
150	M	slight swelling of kidneys	10/15	Gorzinski *et al.*, 1981a (purified 2,4-D)
150	M	increased relative kidney weights		
150	M	increased epithelial cytoplasmic homogeneity of convoluted tubules -diffuse, slight	10/10	
100	M	increased absolute and relative kidney weights		
100	M	increased epithelial cytoplasmic homogeneity of convoluted tubules -diffuse, slight -multifocal, slight -focal, slight	 4/10 5/10 1/10	
60	M	increased absolute and relative kidney weights		
60	M	increased epithelial cytoplasmic homogeneity of convoluted tubules -multifocal, slight -focal, slight -focal, very slight	 2/10 2/10 2/10	
15	M	increased relative kidney weights		
15	M	increased epithelial cytoplasmic homogeneity of convoluted tubules -multifocal, slight	 2/10	
0	M	increased epithelial cytoplasmic homogeneity of convoluted tubules -diffuse, slight -multifocal, slight -focal, very slight	 1/10 1/10 2/10	

(continued)

150	F	slight swelling of kidneys	10 / 15	Gorzinski *et al.*, 1981a
150	F	increased relative kidney weights		
150	F	epithelial cytoplasmic vacuolization of convoluted tubule		
		-multifocal, slight	3 / 10	
		-focal, slight	4 / 10	
		-focal, very slight	3 / 10	
100	F	slight swelling of kidneys	12 / 15	
100	F	increased absolute and relative kidney weights		
100	F	epithelial cytoplasmic vacuolization of convoluted tubule		
		-multifocal, slight	3 / 10	
		-focal, slight	6 / 10	
		-focal, very slight	1 / 10	
60	F	slight swelling of kidneys	2 / 15	
60	F	increased relative kidney weights		
60	F	epithelial cytoplasmic vacuolization of convoluted tubule		
		-multifocal, slight	2 / 10	
		-focal, slight	5 / 10	
		-focal, very slight	3 / 10	
15	F	epithelial cytoplasmic vacuolization of convoluted tubule		
		-focal, slight	1 / 10	
		-focal, very slight	3 / 10	
0	F	epithelial cytoplasmic vacuolization of convoluted tubule		
		-focal, slight	1 / 10	

(continued)

150	M	slight swelling of kidneys	9 / 15	Gorzinski *et al.*, 1981b (technical grade 2,4-D)
150	M	increased absolute and relative kidney weights		
150	M	increased epithelial cytoplasmic homogeneity of convoluted tubules		
		-diffuse, slight	10 / 10	
100	M	slight swelling of kidneys	1 / 15	
100	M	increased absolute and relative kidney weights		
100	M	increased epithelial cytoplasmic homogeneity of convoluted tubules		
		-diffuse, slight	1 / 10	
		-multifocal, very slight	9 / 10	
60	M	increased absolute and relative kidney weights		
60	M	increased epithelial cytoplasmic homogeneity of convoluted tubules		
		-mulifocal, very slight	1 / 10	
		-focal, very slight	7 / 10	
15	M	increased relative kidney weights		
150	F	slight swelling of kidneys	11 / 15	Gorzinski *et al.*, 1981b
150	F	increased relative kidney weights		
150	F	epithelial cytoplasmic vacuolization of the convoluted tubules		
		-multifocal, slight	8 / 10	
		-focal, slight	2 / 10	
100	F	epithelial cytoplasmic vacuolization of the convoluted tubules		
		-mulifocal, slight	2 / 10	
		-focal, slight	6 / 10	
		-focal, very slight	1 / 10	
60	F	epithelial cytoplasmic vacuolization of the convoluted tubules		
		-focal, slight	1 / 10	
		-focal, very slight	6 / 10	
15	F	no effects		

(continued)

45	M	increased absolute and relative kidney weights		ITF, 1983
45	M	increased homogeneity, altered tinctorial properties and fine vacuolization of the cytoplasm in the cortex		
		-moderate	15 / 20	
		-slight	4 / 20	
		-minimal	1 / 20	
15	M	increased homogeneity, altered tinctorial properties and fine vacuolization of the cytoplasm in the cortex		
		-moderate	1 / 20	
		-slight	4 / 20	
		-minimal	9 / 20	
5	M	increased homogeneity, altered tinctorial properties and fine vacuolization of the cytoplasm in the cortex		
		-minimal	10 / 20	
1	M	no effects		
45	F	increased homogeneity, altered tinctorial properties and fine vacuolization of the cytoplasm in the cortex		ITF, 1983
		-moderately severe	1 / 20	
		-moderate	8 / 20	
		-slight	3 / 20	
15	F	increased homogeneity, altered tinctorial properties and fine vacuolization of the cytoplasm in the cortex		
		-moderate	4 / 20	
		-slight	2 / 20	
5	F	increased homogeneity, altered tinctorial properties and fine vacuolization of the cytoplasm in the cortex		
		-moderate	3 / 20	
		-slight	3 / 20	
		-minimal	1 / 20	
1	F	increased homogeneity, altered tinctorial properties and fine vacuolization of the cytoplasm in the cortex		
		-moderate	1 / 20	

TABLE 6. DOSE RESPONSE SUMMARY - THYROID EFFECTS IN RATS

Dose Level	Sex	Effects	Reference
45	M	increase in absolute and relative thyroid weights	ITF, 1983a
15	M	increase in absolute and relative thyroid weights	
15	M	increase in T4 (smaller increase than at 5 mg/kg body weight/day dose)	
5	M	increase in absolute and relative thyroid weights	
5	M	increase in T4	
1	M	increase in absolute and relative thyroid weights	
45	F	no effects on thyroid	ITF, 1983a
15	F	increase in absolute and relative thyroid weights	
5	F	increase in absolute and relative thyroid weights	
1	F	no effects on thyroid	

TABLE 7. EPIDEMIOLOGY PERTINENT TO 2,4-D AND NEUROTOXICITY

Date	First Author	Study Type	Exposure	Reported Findings	Comments
1982	Singer R	case-control	2,4-D and 2,4,5-T	46% of 56 manufacturers showed slowed nerve conduction velocity, compared to 5% of control group; the various measurements were significant.	Exposure based on employment records.
1987	Green LM	cohort, letter	2,4-D and other phenoxy acid herbicides, including 2,4,5-T	Suicides significantly increased in forestry workers.	Subjective assessment of exposure to phenoxy acid herbicides.
1988	Levy CJ	case-control	Agent Orange	Significantly higher rate of posttraumatic stress disorder when compared to unexposed Vietnam veterans.	Based on only six case subjects with chloracne.
1990	Hertzman C	case-control	2,4-D, glyphosate, picloram, formaldehyde, malathion, tebuthiuron, paraquat, diazinon, atrazine, pyrethrum, diquat, and bromacil	Not associated with Parkinson's disease.	Subjective exposure information.
1991	Green LM	cohort	phenoxy acids and other herbicides	Significant increase in suicides in forestry workers.	Subjective exposure assessment.

TABLE 8. EPIDEMIOLOGY PERTINENT TO 2,4-D AND IMMUNOTOXICITY

Date	First Author	Study Type	Exposure	Reported Findings	Comments
1983 Jun 30	USAF School of Aerospace Medicine	cohort	Agent Orange	Statistically insignificant excess of digestive disorder deaths in Ranch Handers.	Cohort of men likely exposed to considerable amounts of phenoxy acid herbicides.
1984	Stubbs HA	cohort	agriculture	Respiratory disease and infective and parasitic disease elevated in California farmers.	No exposure data.
1985	Delzell E	cohort	agriculture	Increased risks of tuberculosis, diseases of the skin and subcutaneous tissue, and external cause mortality among North Carolina farmers.	No exposure information relevant to phenoxy acid herbicides.
1987	Stark AD	cohort	agriculture	Fewer than expected deaths for each major category except accidents in New York.	Large study with considerable power.
1990	Sharma VK	case-control	2,4-D	Patch test gave allergic reactions in three of 30 farmers with contact dermatitis.	Subjective case series.
1990	Thomas TL	cohort	herbicides, riot control substances, and burning agents	Significant excess in digestive diseases in Vietnam Army Chemical Corps veterans.	Small cohort of men likely exposed to phenoxy acid herbicides.
1990	Wolfe WH	cohort	Agent Orange	No immunologic differences in Ranch Handers.	Cohort of men likely exposed to considerable amounts of phenoxy acid herbicides.

TABLE 9. EPIDEMIOLOGY PERTINENT TO 2,4-D AND REPRODUCTION

Date	First Author	Study Type	Exposure	Reported Findings	Comments
1981 Aug 14	Carmelli D	case-control	2,4-D	Significant association between paternal exposure and reproductive problems in an isolated subgroup of wives of young forest / commercial workers in Washington and / or Oregon.	Overall, findings not suggestive of an association between miscarriages and phenoxy acid herbicides.
1983 Jan	Donovan JW	case-control	Vietnam	No reproductive effects in Australian Vietnam veterans.	No exposure information directly relevant to phenoxy acid herbicides.
1984 Aug 17	Erickson JD	case-control	Agent Orange	No overall increased risk for birth defects, but risks of some specific types of birth defects higher among Vietnam veterans more likely to have been exposed to Agent Orange than other Vietnam veterans.	Subjective assessment of exposure to phenoxy acid herbicides.
1990	Dai LC	cohort	herbicides and Vietnam	Significantly increased reproductive problems among North Vietnamese veterans.	Arbitrary exposure assessment with respect to phenoxy acid herbicides.

(continued)

1991	Lerda D	case-control	2,4-D	Associated with significant levels of asthenospermia, necrospermia and teratospermia in sprayers.	Small sample size; exposure based on measured levels of 2,4-D in urine.

letectable (detection limit = 4 µg/L)

subjects showed ND levels.

subjects showed ND levels.

se persons represent the highest 2,4-D urine levels in a total study group of 524 urine samples of which <30% contained 2,4-D levels above the detection limit (0.04 ppm).

dard deviation

rotective apparel was used in this study, but due to lack of other suitable data, it has been included.

subject had an exposure of 5250 µg. This number was excluded because the subject had been spraying extensively with 2,4-D prior to the study.

dard error

kers did not wear protective clothing beyond new boots and gloves. The weather was very hot during the study and workers oftern removed their shirts. In addition, some spillage occurred due to leaky containers.

etectable (detection limit = 0.04 mg/L)

letectable (detection limit = 4 µg/L)

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re were 1 to 7 spray operations spread over 1 to 17 days (spray period).

lard error

one measurement taken; therefore, no range was available.