

**EXPOSURE STUDIES
IN THE USE OF PESTICIDES
IN THE HOME GARDEN AND
FOR LANDSCAPE PEST CONTROL**

FINAL RESEARCH REPORT

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I INTRODUCTION

A large number of households in Ontario use pesticides in one form or another. Common amongst these are garden pesticides used for controlling insects, fungi and weeds. During use, exposure to these pesticides may occur during mixing, application and/or cleaning and repair of equipment. Several pesticides are used extensively in and around the home and, while exposure is probably infrequent, they are of concern because of the large number of persons who may be exposed to these chemicals. The herbicide 2,4-D is one of the most widely used chemicals in the home garden and in landscape weed control and the recent Panel Report (CCT, 1987) pointed to the lack of adequate information on bystander and household exposure to this chemical.

Exposure to pesticides may result from a number of operations that are carried out before, during, and after the application process. Pathways of pesticide exposure include: transport, storage, mixing, loading and application of the pesticides. Exposure may also result from reentry into a sprayed area following the application. Usually the mixing operation results in greater contact with pesticides than any other. Pesticide formulations may be ranked from those which cause the greatest risk of contact during the mixing operation to those with least risk as follows: Dusts > wettable powders > emulsifiable concentrates (high and low volatility) > granular > soluble granules (CCT, 1987). The final operations which would expose applicators to pesticides is the cleaning and repair of equipment, and disposal of empty pesticide containers.

Contrary to label directions, people may re-enter treated areas before minimum re-entry periods have expired. Post-application exposure risk is highest in fruit and vegetable production where hand contact with treated surfaces is greatest. This problem has been recognized for some time and has been the subject of recent extensive reviews (Gunther *et al.*, 1977; Pependorf and Leffingwell, 1982). Toxicological problems concerning re-entry have almost always been associated with organophosphorous pesticides (Kahn, 1980) and this type of bystander exposure is seldom measured for other types of pesticides. Recent studies (Thompson *et al.*, 1984) have suggested that dislodgeability of 2,4-D amine from turf is in the order of 2-8% of the applied amount but the relevance of this to bystander exposure has not yet been determined.

Two general methods of assessing exposure to pesticides have been used in the several existing studies on 2,4-D. These general methods make use of two techniques, one of which is called passive dosimetry and measures actual exposure via sampling of the several possible routes of entry of the pesticide and the other measures actual dose (biological monitoring). The former method makes use of patches or other similar devices attached to parts of the body, personal air samplers and analysis of these then allows an estimation of the exposure on that particular part of the body. By extrapolation and the making of an assumption with regard to uptake via the skin, a dose may then be estimated. This method is useful in estimating exposure to different parts of the body but may miss important areas of exposure should a patch not be used in that location. It also fails to take into account variability in and lower-than-predicted rates of skin absorption. This method also involves several analyses per individual for each time period of exposure.

Biological monitoring depends on a good knowledge of the metabolism and pharmacokinetics of the pesticide in question. Measurement of excretion of the pesticide or a major metabolite will, in the case of certain pesticides, indicate the total dose absorbed by the

body. This method gives a good estimation of the actual dose to which the body is exposed, a useful parameter for assessing possible toxicity, and requires fewer analyses. It also has the advantage that unknown routes of exposure are taken into consideration but it does not allow the verification of the relative importance of these routes.

Following an occupational exposure to 2,4-D amine or ester the majority of the compound will be metabolised to 2,4-D acid in the body and will be excreted in the urine. Exposure to 2,4-D has been measured via this second method in a number of published studies (Frank *et al.*, 1985; Grover *et al.*, 1986; Kolmodin-Hedman and Erne, 1980; Lavy *et al.*, 1982; Lavy *et al.*, 1987; Libich, 1981; Libich *et al.*, 1984; Yeary, 1986). Studies on the pharmacokinetics of 2,4-D acid in humans (Feldman and Maibach, 1974) have indicated that $100 \pm 2.5\%$ of an intravenously administered dose was excreted in the urine in 5 days and that the half-life for excretion was 13 h. After dermal administration of [^{14}C]2,4-D acid ($1 \mu\text{Ci}$; $4 \mu\text{g}/\text{cm}^2$), $5.8 \pm 2.4\%$ of the amount applied was excreted in the urine. Other studies (Sauerhoff *et al.*, 1977) showed a $t_{1/2}$ for the urinary elimination of 17.1 h (range 10.2 to 28.4 h). Most (82%) of the 2,4-D acid was excreted unchanged, but 13% was excreted as conjugates. The clearance of 2,4-D acid from the plasma and the urinary elimination of 2,4-D followed first-order kinetics, which could be described by a one-compartment model. In a study of Canadian farmers (Grover *et al.*, 1986) comparison of urinary acid and patch concentrations of 2,4-D amine showed that there was a positive and significant correlation between the amount deposited on the hands and the amount excreted in the urine. In summary, the half-life for clearance of 2,4-D acid from the body is <24 h. After occupational exposure to 2,4-D, dermal absorption appears to be the major route of entry into the body. These conclusions agree with those of others (WHO, 1984).

The relevant pharmacokinetic studies provide evidence of a reliable and constant relationship between exposure, uptake and urinary elimination of 2,4-D in exposed workers. These studies also suggest that, in workers who use 2,4-D regularly, the amount excreted in the urine over a 24-hour period is a reliable measure of the absorbed systemic dose, provided that they have achieved steady state pharmacokinetics. Extraction of 2,4-D from urine is relatively easy and only a single analysis is needed for the period of observation. Because of the relevance of the dose absorbed and the reduced number of analyses, biological monitoring was used in this research.

The risk associated with exposure to a pesticide or any toxic substance is dependent on both the toxicity of the chemical in question and the dose which enters the exposed person. While many of the questions associated with toxicity and hazard may be addressed through the use of animal tests and procedures, the dose received by users of these substances is often poorly defined. Routinely, exposure studies are required for registration purposes but these usually address exposure under conditions of farm or large-scale use of the pesticide. Very little information is available on exposure of homeowners and the general public to pesticides and even less is known of bystander exposure under these use conditions. This was the basis upon which this study was initiated.

2 EXPERIMENTAL METHODS

2.1 EXPOSURE TO 2,4-D

2.1.1 HOMEOWNERS AND BYSTANDERS

2.1.1.1 OBJECTIVES

The objectives of this study were (1) to determine the effect of 2,4-D formulation and the use of protective clothing on exposure rates of home applicators, and (2) to determine the effect of 2,4-D formulation on exposure of bystanders and concentrations detected in the air.

2.1.1.2 VOLUNTEERS

Forty-four volunteers composed of 22 applicators and 22 bystanders were selected to participate in the exposure study. Bystanders were considered to be persons living within the household but not applying the pesticide. The applicators and bystanders were randomly split into two groups (protective and non-protective apparel) prior to the applications. Two applicators and three bystanders from the non-protective group withdrew from the study for personal reasons, leaving 39 participants. Each applicator agreed to apply a weed and feed fertilizer with 2,4-D in the spring and a liquid formulation of 2,4-D in the fall. One applicator in the non-protective group failed to complete the fall application of liquid 2,4-D.

2.1.1.3 AIR SAMPLING

Air sampling pumps (Gilian Model HFS 113A) connected to absorption tubes via 1.5 metres of TYGON® tubing were calibrated using a bubble flow meter and set to draw air at a rate of 1 L/minute. Absorption tubes were made by filling pasteur pipettes with approximately 2 g of fluorosil (60 mesh) and stopped at both ends with glass wool as described by Frank *et al.*, 1985). Absorption tubes were stored in capped KIMAX test tubes until needed.

Prior to each application, two air sampling pumps connected to absorption tubes were set up 1.5 metres off the ground, one in the front hall way of the house and one outside of the home, downwind of the application site within 3 metres of the property line. Sampling pumps were run from approximately 10 minutes before and up to 30 minutes after each application. All sampling times were recorded.

At the end of each application, one absorption tube was spiked at the field site with 0.22 µg 2,4-D acid dissolved in methanol to serve as a field recovery check. All absorption tubes were returned to their original containers and stored in the freezer until extraction the following day.

2.1.1.4 APPLICATIONS

Eleven applicators and 11 bystanders were selected to participate in the protective group. This group was given verbal instruction regarding measuring, mixing, application, disposal of containers and cleaning of equipment prior to and during the application. Clean overalls, gloves and rubber boots were supplied to the applicator before any equipment or pesticide was handled. The applicator was instructed to keep the additional apparel on until the end of the application.

The nine applicators and eight bystanders in the non-protective or control group wore their typical clothing for pesticide application and were allowed to apply the pesticide as they normally would. Typical clothing worn by the non-protective group included, long pants, running shoes and short sleeved shirts. Gloves or rubber boots were not worn. Only minimal

verbal instructions were given if requested. Apparel and footwear worn during the application was noted.

A granular formulation of fertilizer (10:6:4:) with 1% 2,4-D was used for the spring application. If necessary, a professional 36 inch drop spreader was supplied for the larger properties and a 24 inch drop spreader was used on smaller properties. A liquid formulation of 2,4-D (250 g/L 2,4-D amine) was used for the fall application. All applicators were supplied with a clean hose-end sprayer. One hundred and fifty feet of hose which was rinsed after each use was available to the applicators to reach the far ends of their properties if necessary.

All measuring, mixing and portions of spreading or spraying were videotaped for later visual review. Information regarding product used, rates, area treated, weather, applicator and bystander sex, age and weight was recorded after application.

2.1.1.5 BIOLOGICAL MONITORING

To check for previous exposure to 2,4-D, a morning urine sample was obtained from both the applicator and the bystander on the day of application and was stored in a refrigerator in 500 mL NALGENE bottles until it was picked up that afternoon or evening. Shortly after the application, sub-samples of approximately 100 mL were taken from the morning pre-exposure samples supplied and were spiked with 11 μ g 2,4-D acid dissolved in methanol to serve as field recovery checks. Spikes were stored in 125 mL or 500 mL NALGENE bottles and were returned to the volunteers after application. Volunteers were instructed to store the spiked samples with their day 1 samples either in the refrigerator, in supplied styrofoam coolers or soft sided cooler bags with frozen ice packs.

Immediately following application, both the applicator and the bystander were instructed to collect all urine for a consecutive 4 day period. A minimum of 4 clearly labelled 2 L NALGENE bottles were supplied to each volunteer, one or more for each day. For ease of collection, volunteers were instructed to void all samples immediately following application until 8:00 or 9:00 am the following day. This day-1 period varied from 12 to 24 hours depending on the time of application. The day-1 sample period for each volunteer was recorded. Day-2 to day-4 samples were collected over 24 hour periods and picked up each day at the home or work address.

Upon arrival in the laboratory, the volumes of all samples (pre-exposure, spike, day 1, 2, 3 and 4) were measured and 1 ml sub-samples were taken from day 1, 2, 3 and 4 samples and analyzed for urinary creatinine. Urinary creatinine is a waste product of creatine which is necessary for muscle metabolism, and it is formed by an irreversible reaction at a fairly constant rate. It is commonly used as an index of the completeness of 24-hour urine collections.

2.1.2 PROFESSIONAL APPLICATOR AND BYSTANDER EXPOSURE

2.1.2.1 OBJECTIVES

The objectives of this study were to measure the daily exposure of professional applicators over a 2 week period and under conditions of use in Ontario, and to measure concentrations of 2,4-D in air samples and urine samples supplied by bystanders who received a professional application of 2,4-D to their property.

2.1.2.2 PROFESSIONAL APPLICATORS

2.1.2.2.1 Subjects

A professional lawn care company in Ontario was asked to participate in the study. Agreements from managers/owners from two locations were obtained. Five lawn care technicians from one area and seven technicians (including one mixer/loader) from another location agreed to participate in the study.

2.1.2.2.2 Products used

Records of the amount of 2,4-D grams active ingredient (A.I.) sprayed were kept for each technician throughout the 14 day period. Technicians from group 1 sprayed a mixture of 2,4-D amine/mecoprop (118:125 g/L). Volunteers from group 2 applied a mixture of 2,4-D amine/mecoprop/dicamba (200:10:18 g/L). All technicians were asked to supply information regarding application procedures, and personal information such as age and weight.

2.1.2.2.3 Biological monitoring

Prior to the commencement of spraying in the spring (Group 1), and in the fall (Group 2), a pre-exposure urine sample was collected from each participant on the first day of the study. Daily samples were collected from 11 technicians for a period of 14 days including week-ends or days off. One technician supplied samples for a period of 7 days. The technicians were instructed to store their urine samples in the supplied cooler bags with frozen ice packs while at work and in the refrigerator while at home overnight. Frozen ice packs were supplied each day of the study.

Field-recovery spikes of 11 μg 2,4-D acid dissolved in methanol in approximately 100 mL of urine were processed to verify percent recovery of 2,4-D in 1, 2 and 3 day samples that were not possible to collect (i.e. long weekend, holiday). If possible, all samples were picked up each day and extracted that same day. Sub-samples of 1 mL were taken from each daily sample provided by the technicians for use in creatinine analysis.

2.1.2.3 BYSTANDER EXPOSURE

2.1.2.3.1 Subjects

Ten volunteers were selected in the Guelph area to participate in the bystander exposure portion of the study. Three volunteers withdrew from the study for personal reasons. All professional applications were set up to be completed in one day by one applicator. The remaining three applications were completed the following summer.

2.1.2.3.2 Air sampling

Air sampling methods were conducted as previously described (Section 2.1.3) Sampling pumps were run from approximately 10 minutes before and up to 15 minutes after each application.

2.1.2.3.3 Applications

Once air samplers were running, the technician was free to apply the pesticide in the manner in which they had been trained. A mixture of 2,4-D amine/mecoprop (110:115 g/L) was applied. Portions of the spraying were videotaped for later visual review. After each application, the property was measured and a sign was posted indicating pesticide application. Tank dip measurements recorded by the technician were used to estimate the amount of pesticide sprayed on each property.

2.1.2.3.4 Biological monitoring

Urine sampling procedures were conducted as those described in Section 2.1.5. However, volunteers were instructed to store the spiked samples with their day-1 samples either in the refrigerator or in soft sided cooler bags with frozen ice packs.

2.2 CONTROLLED EXPOSURE

2.2.1 OBJECTIVES

The aim of this study was to simulate a park or playground situation where people may be exposed to the herbicide 2,4-D; to measure that exposure in relation to the type of clothing worn and the amount of mechanically dislodgeable 2,4-D at the application site; and to determine appropriate re-entry periods for the general public following application of pesticides to private and public areas.

2.2.2 VOLUNTEERS

Volunteers were chosen from faculty, staff and students at the University of Guelph. Ten volunteers, consisting of two females, aged 22 and 25, and eight males ranging in age from 22 to 55 participated in the exposure session 1 hour following the pesticide application, and 24 hours following pesticide application, ten volunteers consisting of two females, aged 25 and 31, and eight males aged 22 to 61 took part in the study. Prior to both studies, the volunteers were split into two groups. Five of the participants wore long pants, a t-shirt, socks and closed footwear. The other five wore shorts and a t-shirt and were barefoot. All volunteers were supplied with written information outlining the possible risks they would be taking to participate in the study. It was estimated that no higher than 1 mg total dose would be possible. The protocol was appraised and approved by the University of Guelph Ethical Review Board and Consent forms were signed before the initiation of the study.

2.2.3 APPLICATION

The area of turf used was mowed three days in advance of each study to level out the grass surface. Ten areas of 2 m by 15 m were marked out side by side with a bright yellow rope pegged into the ground. Five areas of 1 m by 1 m were marked out for the dislodgeability portion of the study. A professional lawn care company representative of the industry in Ontario was asked to apply the pesticide on both dates. A mixture of 2,4-D amine/mecoprop/dicamba (190:100:18 g/L) was used at a rate of 1.0 kg a.e.(acid equivalent)/ha. Theoretically, 3.3 g of 2,4-D a.e. were present in each 2 by 15 m plot and 0.11 g a.e. in each 1 m by 1 m plot. The plots were sprayed cross wise so that any overlap in the spray pattern would be identical in each plot.

2.2.4 EXPOSURE

The re-entry periods tested in this study were 1 hour and 24 hours following 2,4-D application. The same area of turf was used for both exposure periods which were conducted 1 month apart under similar weather conditions. No rainfall occurred on either exposure day.

The volunteers arrived at the spray site between the period of 12:00 and 12:15 p.m. and were randomly assigned to plots. At 12:30 (1 hour and 24 hours after the completion of the spray application) they were asked to step into their plots and begin the 60 minute exposure session. The volunteers were instructed to walk on the turf for a period of 5 minutes and then sit or lie on the area for 5 minutes and to continue in this fashion for 50 more minutes. Each person was asked to cover the largest area possible in the plot while walking and to expose the greatest area of their body while lying or sitting on the plot. Volunteers sat on six different areas of the plot to facilitate maximum exposure. One volunteer in the 1-hour exposure session removed his shirt during the last 30 minutes of the study. The whole exposure period was videotaped. Following the 60 minute exposure, the participants were allowed to wash their hands and were served a picnic lunch on an adjacent unsprayed area of turf.

2.2.5 DISLODGEABLE RESIDUES OF 2,4-D

During the 1 hour exposure period, dislodgeable residues were determined by vigorous wiping of five, 1 m² plots as previously described by Thompson *et al.* (1984). A pair of running shoes was covered with disposable polyethylene plastic bags and a double layer of cheesecloth, tied above the ankle. The cheesecloth was moistened with distilled water and the sampling was performed by scuffing backwards and forwards across the grass in a 1 m² area for 1 minute while wearing the covered shoes. The cheesecloth was removed from the shoes, and the excess unexposed material was cut away. The cheesecloth sample was immediately immersed in acetone and transported to the laboratory for extraction. Dislodgeable residues of 2,4-D were analyzed as described by Thompson *et al.* (1984).

2.2.6 BIOLOGICAL MONITORING

Urine sampling procedures were conducted as described in Section 2.2.3.4.

2.3 PERCUTANEOUS PENETRATION OF 2,4-D ACID AND 2,4-D DMA IN HUMAN VOLUNTEERS

2.3.1 OBJECTIVES

The objectives of this study were to: 1) determine the difference in percutaneous absorption between 2,4-D acid and 2,4-D dimethylamine salt (DMA) following dermal administration to the dorsum of the hand of five male volunteers; 2) to determine the relationship between the amount of each formulation absorbed and the amount washed off the hand 6 hours following administration; 3) to determine half-lives of excretion for each formulation; and 4) to determine if 96 hour urine collection is sufficient to calculate absorbed dose following a human exposure to 2,4-D.

2.3.2 SUBJECTS AND APPLICATION

Five healthy male volunteers aged 27 to 48, weighing 75 to 100 kg participated in both experiments. A solution of 10 mg of 2,4-D dimethylamine salt, technical formulation (2,4-D DMA 710, 710 g/L acid equivalent (a.e.), supplied by May and Baker) dissolved in 40 µL

distilled water was applied to an area of 9 cm² on the hand dorsum. This dilution was chosen as it is representative of the concentration of 2,4-D contained in products registered for domestic use in Ontario. The solution was pipetted onto the area and evaporated by gentle blowing. One month following the first application, a second application of 10 mg analytical grade 2,4-D acid (99.5% purity) dissolved in 500 µL acetone was pipetted onto the back of the hand of each volunteer and was evaporated by gentle blowing. The area of skin was not protected and subjects were instructed to avoid all contact of the application site with clothing or other materials.

This study was conducted using water as a carrier for the 2,4-D amine to parallel a practical field situation. It is more relevant to determine the dermal absorption of 2,4-D formulated product in its typical carrier (i.e. water). The acid formulation of 2,4-D dissolved in acetone was used in the second study to determine if results from previous studies could be reproduced, and if so, to determine the practical difference in absorption of the formulated product in water to that of the acid formulation in acetone.

To determine the amount of 2,4-D which could be removed from the skin, six hours following the application, the marked area was washed with 100 ml distilled water and scrubbed with a toothbrush. The wash water was then analyzed for 2,4-D. Recovery spikes of 10 mg 2,4-D acid or 2,4-D DMA were conducted for each application and used to correct for incomplete recovery. This time interval was chosen to simulate a practical field situation. In previous dermal absorption studies (Feldman and Maibach, 1974), the pesticide has been left on the skin for 24 hours. Most homeowner applicators wash their hands immediately following a pesticide application. Professional applicators who spray pesticides on turf may wash their hands at lunch hour and dinner or every 6 hours. Therefore, a large percentage of pesticide on the skin may be washed off and a lower amount available for absorption.

2.3.3 *BIOLOGICAL MONITORING*

To check for previous exposure to 2,4-D, a morning urine sample was obtained from the volunteer on the day of application. Sub-samples of 100 mL were taken from the morning pre-exposure samples supplied and were spiked with 11 µg 2,4-D acid dissolved in methanol to determine recovery. Spikes were stored in 125 mL NALGENE bottles and returned to the volunteers following the dermal application. Volunteers were instructed to store the spiked samples with their day ½ and day 1 samples in soft sided cooler bags with frozen ice packs.

Immediately following application, the participant was instructed to collect all urine for a consecutive 6 day period. The first day, urine was collected in two 12 hour periods. The remaining 5 days, urine was collected as 24 hour specimens except for one volunteer who collected for 12 hour periods for the entire study. Two L NALGENE bottles were supplied to each volunteer, one or more for each day.

Upon arrival in the laboratory, all samples (Day 1, 2, 3, 4 and 5) were measured and 1 mL sub-samples were taken and analyzed for urinary creatinine to verify compliance with complete urinary collections.

2.4 EXPOSURE TO 2,4-D - ANALYTICAL METHODS

2.4.1 CHEMICALS

All solvents were of pesticide grade (Caledon Laboratories Ltd., Georgetown, Ont., Canada). The analytical standard of 2,4-D was obtained from Sigma Chemical Company (P.O. Box 14508, St. Louis, Mo 63178). Polyclonal antibodies, the 2,4-D^[3H] glycine conjugate, buffers, and other reagents, were supplied by Dr J.C. Hall, Department of Environmental Biology, University of Guelph, Guelph, Ont. The "Res-I-Mune" ELISA kit, manufactured by Immunosystems Inc., Biddeford, Maine 04005, was supplied courtesy of Biotech-Transfer Consulting Inc., Waterloo, Ont.

2.4.2 URINE SAMPLES

Human urine was collected from a male donor over a 24 hour period, pooled, and stored at 4°C.

2.4.3 EXTRACTION

2.4.3.1 WATER

One litre of water containing 2,4-D was acidified with 2.5 ml of 1:1 H₂SO₄ and extracted twice with 50 mL of diethyl ether. The ether extracts were combined and dried with Na₂SO₄ and evaporated to dryness.

2.4.3.2 URINE

Urine samples (50 mL) were first hydrolysed with 250 mL of 0.1 N NaOH for 40 min at 70° and washed three times with 50 mL of dichloromethane/hexane (20:80, v/v). The aqueous portions of each urine extraction were then processed as the waters.

2.4.3.3 ESTERIFICATION

The herbicide residues were esterified with boron trifluoride in methanol (14% BF₃) at 90°C for 30 min. The methyl esters of the herbicide were extracted with three x 5 ml petroleum ether and 25 ml distilled water was added to the mixture to remove the excess boron trifluoride. The petroleum ether extracts were dried with anhydrous Na₂SO₄ and the total volume adjusted to 5 mL by evaporation. Isooctane was added as a keeper.

2.4.3.4 GAS CHROMATOGRAPHY

Either a Tracor 550 and a Varian 3700 gas chromatograph equipped with an electron capture (⁶³Ni) detector was used to quantitate herbicide residues. The gas chromatographic column was a glass column, 2 mm I.D. x 2 m in length, packed with 3% OV 17 on GasChrom Q. The temperature was kept isothermal at 180 the detector at 300 and the injector at 200°C. The peak heights obtained in each fortified sample or unknown sample were quantitated relative to peak heights obtained for a known quantity of 2,4-D (a stock solution of 1000 µg/L was diluted to a working concentration of 0.1 µg/L).

2.4.4 RADIOIMMUNOASSAY (RIA) PROCEDURE

A serial dilution of 100 µl of standard or sample was transferred into a 1.5 mL microcentrifuge tube (Fisher Scientific, Don Mills, Ont.). Control tubes received 100 µl of non-fortified sample solution. Incubation mix (300 µl per tube) consisting of one part deionized

water, one part inert serum, 12 parts PBS (phosphate buffered saline), and sufficient radiolabel to yield 10,000 cpm per assay was added to each tube. Antisera diluted in PBS (1:100) was added to the tubes (100 μ l per tube). One set of control tubes did not receive antisera for determination of non-specific binding and a second set of control tubes received antisera only for maximum binding of radiolabel (B_0). The contents of the tubes were mixed thoroughly on a vortex mixer followed by a 2 hour incubation at 4°C. The antibody-bound radiolabel fraction was precipitated by adding 0.5 mL of a 90% $(\text{NH}_4)_2\text{SO}_4$ solution, mixing, and incubating for one hour at 4°C. The precipitate was centrifuged (12,000 g) for 5 min and the supernatant was discarded. The pellet was washed once with a 0.5 mL portion of a 50% $(\text{NH}_4)_2\text{SO}_4$ solution. The tubes were re-centrifuged and the supernatant discarded. The pellet was dissolved in two 300 μ l aliquots of deionized water and was transferred to 6 mL scintillation vials. Each vial received 4 mL of scintillation cocktail (Aquasol 2). The scintillation vials were assayed for radioactivity on a Packard Tri-Carb 460C liquid scintillation system. All results were corrected for non-specific binding. Values for standards were divided by B_0 and were plotted against the log of the herbicide concentration ($\mu\text{g/L}$). The quantity of the herbicide in the unknown sample was calculated based on the standard curve.

2.4.5 ENZYME LINKED IMMUNOASSAY (ELISA)

The Res-I-Mune kit was used according to instructions. Briefly, 4 drops (160 μ l) of sample or standard 2,4-D were put in antibody pre-coated tubes, followed by 4 drops of the 2,4-D "enzyme conjugate". The tubes were allowed to incubate for 10 minutes at room temperature before rinsing the unreacted mixture away using 4 water rinses. Four drops of substrate followed immediately by 4 drops of chromogen were added to the nearly dry tubes and the tubes incubated for 5 minutes at room temperature. The tubes were examined visually by observing the blue colour produced. In order to read the results on a spectrophotometer, the reactions in each tube were stopped by adding 4 drops of the supplied acid solution. The yellow colour produced in the tubes, was read using a Varian Superscan 3 ultraviolet visible Spectrometer at 450 nm. The absorbances obtained were graphed against the concentration ($\mu\text{g/L}$) of 2,4-D to obtain a standard curve. Unknowns and standards were analyzed in duplicate with the unknowns being quantitated on the basis of the standard curve.

2.4.6 RESULTS

The standard curves obtained for the liquid-liquid extraction of 2,4-D from water are given in Figure 1. The concentrations for each set of fortified samples analyzed ranged from 0.05 to 10,000 $\mu\text{g/L}$. The results indicated a distinct linear relationship with the correlation coefficients of the lines for distilled water and fortified waters at 0.9997 and 0.9983 respectively. This indicates that a conventional liquid-liquid extraction technique was suitable for determining 2,4-D in water over a wide range of concentrations. It should be noted that a level as low as 0.005 $\mu\text{g/L}$ was tested but was determined to be below the minimum detectable level. The sensitivity of the electron capture detector to the two chlorine atoms on the methylated 2,4-D is such that low levels such as 0.1 $\mu\text{g/L}$ can be detected. Since the water extractions involved a concentration step equivalent to 200-fold, the detection limit for 2,4-D in water by conventional gas chromatography is 0.0005 $\mu\text{g/L}$. Recoveries were determined by fortifying water prior to

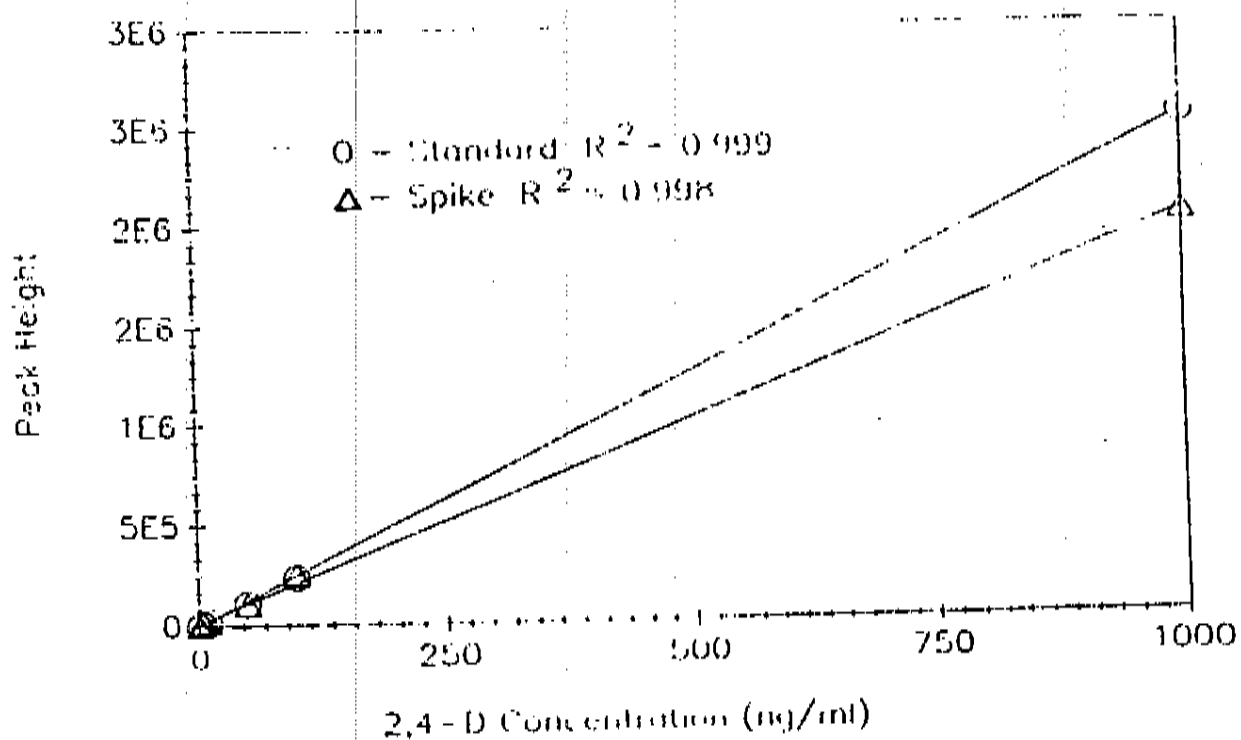


Figure 1 Standard curves for the determination of 2,4-D in water by Gas Chromatography. Each point represents the mean of two determinations

extraction with 2,4-D at levels of 1.0, 10, 100, and 1000 µg/L; the recoveries and the standard deviation for six replicates in brackets were 86(12), 92(8), 97(9.2) and 101(7.5) respectively.

The results for the analysis of water for 2,4-D by radioimmunoassay are given in Figure 2. All assays for standards and samples were performed in triplicate and the means are graphed. As indicated in Figure 2, RIA was suitable for the determination of 2,4-D in water over a wide concentration range of 10 to 10,000 µg/L. The assay showed a linear relationship with a correlation coefficient of 0.9871. Comparatively, water samples were analyzed that were fortified in the range of 10 to 10,000 µg/L and the results showed a similar linear relationship with a correlation coefficient of 0.9525. At levels of 10 µg/L or less, the sensitivity of the assay is somewhat questionable. This indicates that the RIA assay is suitable for determining levels of 2,4-D in water at a range (10 - 10,000 µg/L) slightly less than that for liquid-liquid extraction (0.05 - 10,000 µg/L). In spite of this narrower range of determination, the RIA assay was used directly without a concentration step. By adding a concentration step, such as that used in the liquid-liquid extraction, or using some solid adsorbents such as Sep-Paks, the RIA could be used to determine significantly lower levels of 2,4-D in water.

The Res-I-Mune kit test results are given in Figure 3. The standard curve for the kit also gave a linear relationship (correlation coefficient of 0.9882) for absorbance versus concentration but at a narrower range of concentration (1.0 - 100 µg/L). This range could be extended to

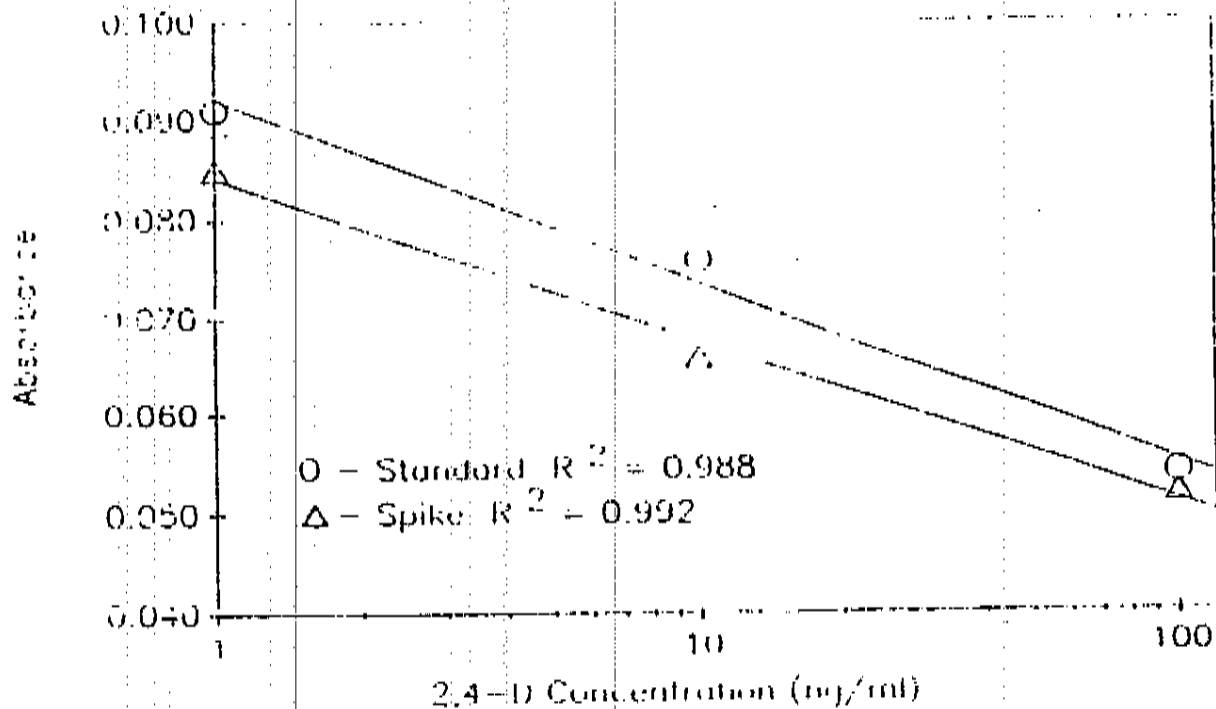


Figure 3 Standard curves for the determination of 2,4-D in water by Res-I-Mune (ELISA). Each point represents the mean of two determinations

coefficient of 0.9234.

Each of the three assay techniques were tested using fortified water samples as unknowns. The results for the analyses obtained by each of the three methods is given in Table 1. As indicated, both the RIA and GC methods show excellent recovery in the range of 1.0 to 10,000 µg/L with results obtained by RIA being slightly better than those obtained by GC. The analysis of a 10,000 µg/L fortified water sample by GC was quantitated to be 14,000 µg/L which represents a recovery of 140%. The number is higher than anticipated but it is important to note that this concentration is far greater than the normal detection level of 1.0 µg/L or quantities used in the normal environmental range of 0.1 to 1.0 µg/L.

The results obtained by the ELISA technique are more remarkable. The 100 µg/L fortified sample was quantitated to be 101 µg/L but quantitation at 1.0 and 10.0 µg/L gave results of 2.0 and 21 µg/L respectively. These results represent 200% recovery and are the mean of two determinations. The slope of the line is critical and any minor change in absorbances could dramatically alter the results. The linearity of the kit is a function of the amount of antibody in the tube. When 2,4-D concentrations greater than 100 µg/L are used, there is insufficient antibody to give positive results.

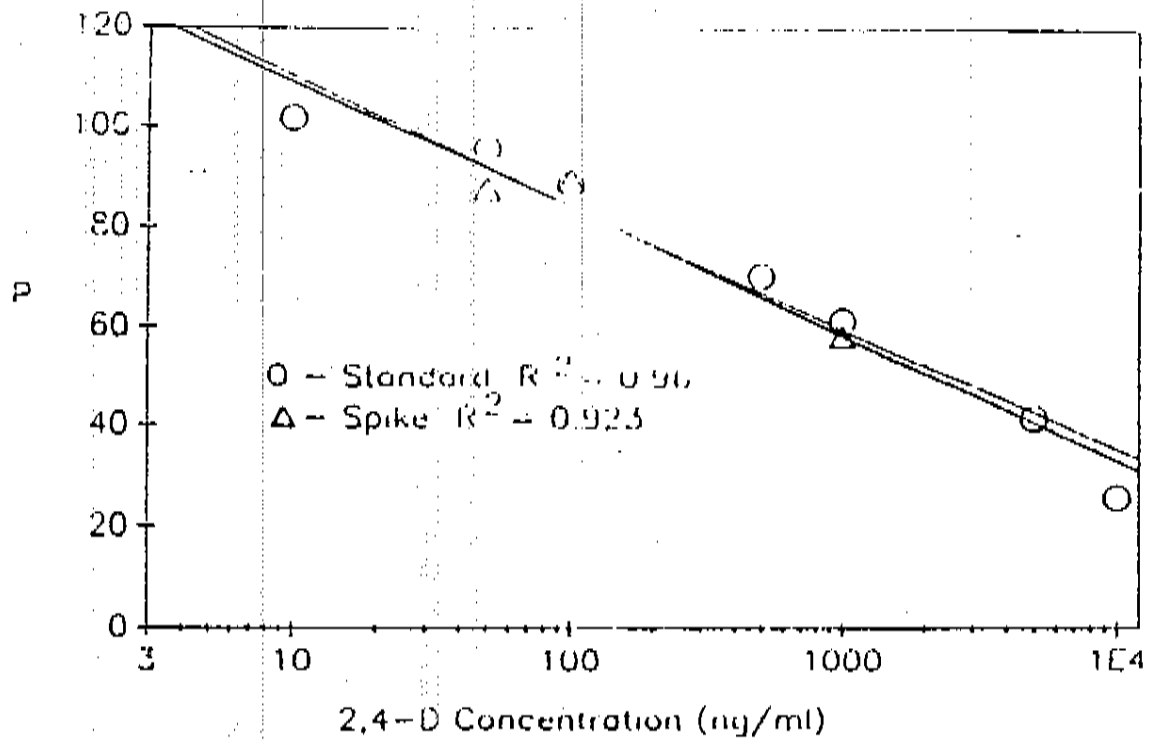


Figure 4 Standard curve and spiked recovery for the determination of 2,4-D in urine by radioimmunoassay (RIA). Each point represents the mean of three determinations

	RIA ¹	GLC ²	ELISA ³
(Fortification level $\mu\text{g/L}$)	2,4-D $\mu\text{g/L}$		
1.0	NA ⁴	NA	2.0 (200)
10	8.7 (87) ⁵	8.3 (83)	21. (210)
100	95. (95)	100. (100)	110. (110)
1000	1000. (100)	820. (82)	NA
10000	10600. (106)	14000. (140)	NA

1. RIA: Radioimmunoassay
2. GC: Gas liquid chromatography
3. ELISA: Enzyme linked immunosorbent assay

1. RIA: Radioimmunoassay
2. GC: Gas liquid chromatography
3. ELISA: Enzyme linked immunosorbent assay
4. ND: Not detected
5. NA: Not analyzed
6. Percent recovery is indicated in brackets

Table 7 Applicator and bystander exposure in relation to grams of weed-and-feed 2,4-D (A.I.) applied under conditions of non-protective application.

		2,4-D Urine (total $\mu\text{g}/\text{person}$ in 4 days)		Creatinine in urine (average 4 day, $\text{mmole}/24$ hours)		2,4-D Air ($\mu\text{g}/\text{m}^3$)	
1a	1200	169	ND	19.8	11.1	ND	ND
2b	800	ND	ND	15.9	7.3	ND	ND
3c	500	ND	ND	9.9	7.5	ND	ND
4d	1200	ND	ND	12.9	8.6	ND	ND
5e	300	ND	ND	17.2	11.3	ND	ND
6f	200	ND	ND	14.5	8.2	ND	ND
7g	200	ND	--	9.9	--	ND	ND
8h	400	ND	ND	12.4	4.6	ND	ND
9i	150	ND	ND	17.4	8.1	ND	ND
\bar{X}	550	18.8	ND	--	--	ND	ND

ND - not detectable $< 4 \mu\text{g}/\text{L}$

No bystanders in this group showed exposure to 2,4-D. One positive indoor air sample was found in the non protective applications (Volunteer #1a, $0.01 \text{ mg}/\text{m}^3$). Once again, this did not result in measurable exposure of the bystander and may have been as a result of accidental contamination during handling.

Lack of protection of the hands and forearm obviously resulted in a greater incidence of exposure in the unprotected group, however, highest exposure was consistently associated with spills or accidental contamination of the skin. Previous studies (Davies, *et al.*, 1983) have shown that 85% of potential exposure to diazinon during yard applications could be eliminated by protecting the applicators hands. Spills from the original container could have been avoided through the use of a better design to allow for easy pouring and the use of a firmer plastic for construction. Leaks of concentrate from the hose-end sprayer could be reduced through better design of seals between the sprayer and the pesticide container and through the use of a freely rotating coupling to the end of the hose.

It appears that the use of protective apparel for application of a granular formulation of 2,4-D does not reduce exposure in the applicator. However, an obvious difference occurs when applying 2,4-D liquid. The use of rubber gloves and possibly overalls and rubber boots, when pouring, applying and cleaning the equipment reduced exposure.

3.2 PROFESSIONAL APPLICATOR AND BYSTANDER EXPOSURE

3.2.1 APPLICATOR EXPOSURE

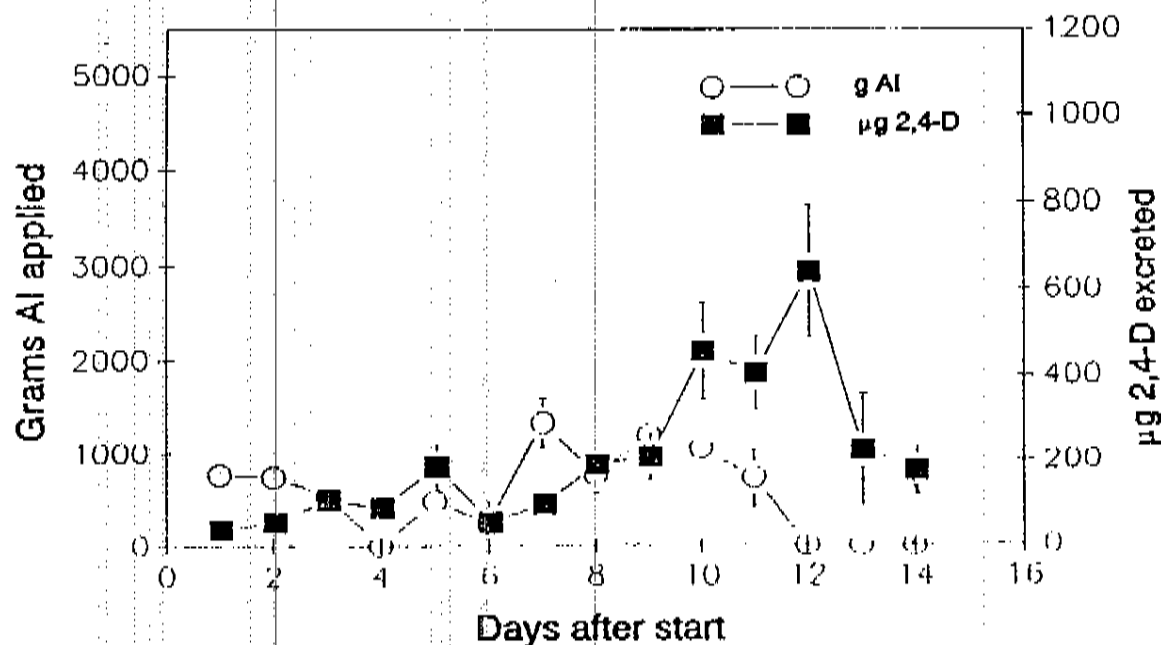


Figure 5 Mean exposure of Group 1 to 2,4-D in relation to amount of A.I. used. Vertical lines indicate the SE of the mean.

Mean exposure in group 1 was analyzed in relation to actual amount of 2,4-D excreted in the urine and the amount of 2,4-D excreted in relation to the amount of creatinine excreted on each day (Figure 5). Use of creatinine excretion as a correction factor did not greatly change the pattern of exposure and, in both analyses, 2,4-D excretion showed two peaks. These peaks of excretion lagged behind the amounts of A.I. used on each day by a period of 2-4 days. Due to high variability between individuals in this group, linear regression analysis failed to show high correlations between the total amount of 2,4-D sprayed and the total excreted, either on a daily basis or over the entire period of the study. The raw data and the individual applicator graphs are given in Appendices B and C.

Group 2 showed essentially the same trend as group 1 both in total 2,4-D excreted or 2,4-D excreted per mmole of creatinine (Figure 6) but the highest mean exposure in the group was lower than in group 1, despite the fact that more 2,4-D (in total) was applied by group 2.

In addition to the applicators in groups 1 and 2, exposure was measured in a manager who had the daily responsibility for mixing and loading the spray trucks. Although this person was not involved in actual spraying, he did handle the largest amount of pesticide of any person in the study. The exposure of the mixer/loader (Figure 7) was lower than the mean of the spray crews from the same group (Figure 6), despite the considerably higher amount of 2,4-D concentrate handled.

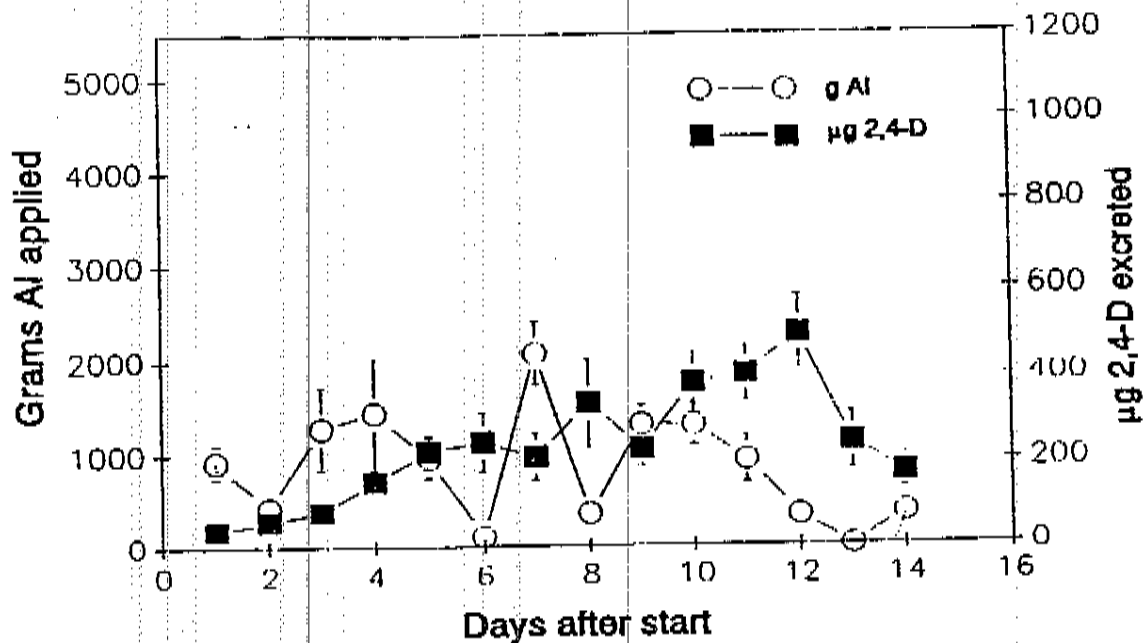


Figure 6 Mean exposure of Group 2 to 2,4-D in relation to amount of A.I. used. Vertical lines indicate the SE of the mean.

There was high variability in the exposure of individual sprayers in relation to the amount of 2,4-D A.I. applied. Comparison of total 2,4-D excreted to the amount excreted per mmole of creatinine suggested some incomplete samples, but, on the whole, compliance with collection protocols was good. Total exposure was poorly correlated with total amount of A.I. used (Table 7, Figure 8) and exposure data corrected for creatinine values also failed to show correlation with A.I. used. It was not possible to observe each sprayer during all of their regular operations for the 14 day period, but it is likely that 2,4-D dose is correlated with personal work practices and precautions taken to decrease exposure. In other words, poor technique leads to higher exposures.

The highest single-day exposure to 2,4-D in any of the sprayers was 1.108 mg. This is about 1/19 of the ADI suggested by the World Health Organization which is 0.3 mg/kg/day or 21 mg/day in a 70 kg person, (WHO, 1984). In all other cases, exposure was lower and, if averaged over the two week period of the study, gave higher safety factors when compared to the ADI. All average exposures fell within the range previously reported by Yeary (1986). These results suggest that exposure of spray applicators to 2,4-D under these circumstances does not present an unacceptable risk. However, it should be noted that exposure was variable from one individual to another and the correlation between amount of pesticide used and exposure was very poor. These observations suggest that personal work habits and hygiene may be the major factors which affect exposure. Good training, consistent use of personal protective equipment and adherence to a code of good spray practice may reduce exposure still further.

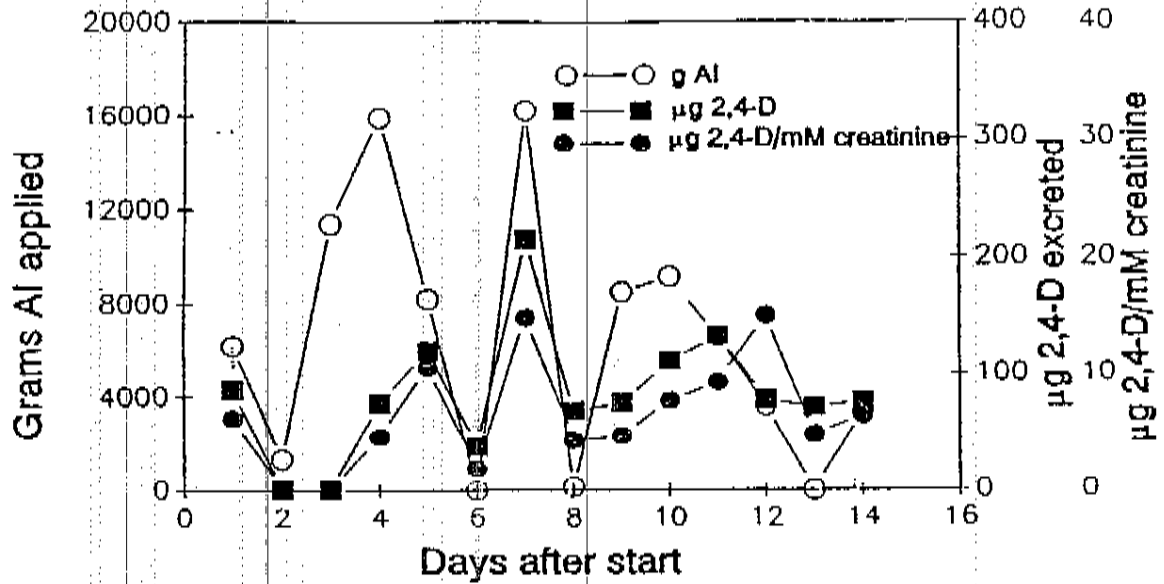


Figure 7 Exposure of mixer/loader to 2,4-D (total and per mmole of creatinine excreted) in relation to amount of A.I. used.

3.2.2 BYSTANDER EXPOSURE

No exposures were detected in volunteers who had a professional application of 2,4-D to their property (Table 8). All creatinine values were above or fell within the normal range for adult males (7.0-18.0 mmole/24 hours) and adult females (5.0-16.0 mmole/24 hours), indicating complete sample collection.

Air samples taken both inside and outside the home downwind of the application showed

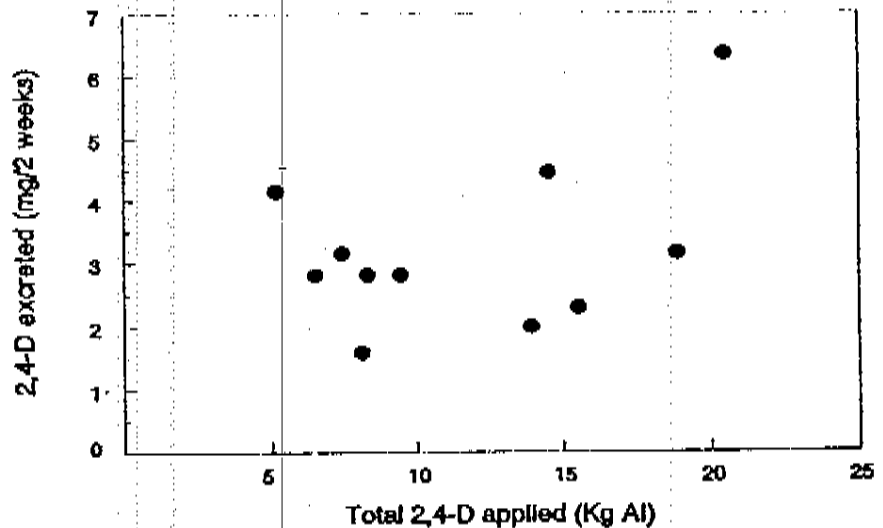


Figure 8 Relationship between total 2,4-D applied and total excreted in professional applicators

no detectable residues of 2,4-D. The lack of detection of any 2,4-D in the bystanders' urine samples or in air samples suggests that exposure to 2,4-D via this use-pattern is very low and presents a minimal risk.

Table 7 Total 2,4-D use and excretion in professional applicators and a mixer/loader (M/L).

Vol #	Total 2,4-D applied (g) ¹	Total 2,4-D excreted (µg)	Total µg/mmoles creatinine	Total µg/g A.I.	Average dose (µg/kg/day)	Fraction of ADI
1	51282	4127	419.2	0.80	4.4	1/68
2	8300	2812	161.6	0.34	2.8	1/107
3	8101	1574	170.1	0.19	1.7	1/176
4	9399	2782	214.1	0.30	3.3	1/90
5	6577	2800	249.9	0.43	4.9	1/61
6	7400	3128	226.0	0.42	2.8	1/107
7	13960	2011	145.1	0.14	2.1	1/143
8	20480	6345	344.9	0.31	5.5	1/55
9	14500	4421	231.4	0.30	3.1	1/97
10	18880	3125	267.9	0.17	3.7	1/81
11	15480	2289	121.4	0.15	2.0	1/150
M/L	90700	1141	90.0	0.01	0.9	1/333
Mean ²	11655	3219	—	0.32	3.3	1/103

1 R² for the regression of total µg excreted vs total g applied = 0.172

2 Mean does not include values for mixer/loader.

Volunteer #	2,4-D applied g A.I.	Total 2,4-D in urine (μg) ¹	Creatinine ³	2,4-D $\mu\text{g}/\text{m}^3$	
				Inside	Outside
1	9.43	ND ²	7.80	ND	ND
2	37.63	ND	10.1	ND	ND
3	8.17	ND	11.7	ND	ND
4	147.2	ND	6.30	ND	ND
5	48.15	ND	16.7	ND	ND
6	41.63	ND	13.0	ND	ND
7	15.83	ND	16.0	ND	ND
8	21.52	ND	11.2	ND	ND
9	7.90	ND	15.0	ND	ND
10	59.05	ND	18.1	ND	ND

1 2,4-D μg in combined 4 day urine sample corrected for field recovery

2 ND - not detectable $< 4 \mu\text{g}/\text{L}$

3 average four day creatinine value (mmole/24 hr) sample collection.

3.3 CONTROLLED EXPOSURE

3.3.1 DISLODGEABLE RESIDUES OF 2,4-D

Dislodgeable residues of 2,4-D determined 1 hour and 24 hours following application (Table 9) were similar to those found by Thompson *et al.* (1984) who found that less than 6% could be dislodged immediately after spraying at a rate of 2.24 kg a.e./ha and 4.5% when sprayed at a rate of 1.0 kg a.e./ha. Although residues determined in this study were slightly higher, they showed a similar rapid decrease over time. Higher dislodgeable residues may have been due to spraying techniques used, weather conditions and the composition of the turf surface. It should also be noted that a professional applicator applied the pesticides and the researchers had no control over the rate. This may have resulted in higher than recommended rates applied to the plots. Results of these dislodgeability studies cannot be directly statistically compared because experiments were conducted 1 month apart. These results do however, allow for a comparative estimation of human exposure based on amount of dislodgeable residues at the time of exposure.

Similar comparisons have been attempted by other researchers. Zweig *et al.* (1985) determined the relationship between dermal pesticide exposure by fruit harvesters and dislodgeable foliar residues of captan, vinclozolin, carbaryl, and methiocarb. A positive correlation between these two parameters was found and log-log regression analyses were

essentially linear between dislodgeable foliar residues and dermal dose rates. The authors proposed that a rough first approximation of dermal exposure rate for fruit harvesters based on dislodgeable foliar residues (DFR) can be calculated using the following expression:

$$\text{Dermal Exposure Rate (mg/hr)} \approx 5 \times 10^3 \times \text{DFR}$$

This transformation suggests a method for obtaining exposure rates of fruit harvesters in order to obtain safe re-entry periods without involvement of human subjects. However, this equation does not account for variation in percutaneous penetration of different pesticides and does not allow for the calculation of total dose, which is more relevant to human health risk assessment.

Plot	Dislodgeable residue - 1 hour		Dislodgeable residue - 24 hours	
	mg/m ²	% of applied	mg/m ²	% of applied
1	10.030	9.118	1.166	1.060
2	10.952	9.956	1.161	1.055
3	8.200	7.455	1.024	0.9309
4	4.904	4.458	1.080	0.9818
5	8.144	7.085	1.156	1.051
Average	8.446 ± 0.927	7.614 ± 0.949	1.117 ± 0.028	1.016 ± 0.026
Control	ND ¹	NA ²	ND	NA

1 Non-detectable < 5 µg/L

2 Not applicable

A herbicide which may be highly dislodgeable from leaf or plant surfaces (which indicates high dermal exposure rates) may be poorly absorbed through human skin thus reducing systemic dose. Studies conducted by Feldmann and Maibach (1974) indicate that, of 12 pesticides tested, carbaryl was almost completely absorbed (73.9%) following topical administration to the ventral forearm, and diquat showed only slight penetration. All other compounds tested ranged between 5 and 20% absorption. These absorption factors will dramatically affect total dose absorbed in humans and must be considered when establishing re-entry periods.

3.3.2 HUMAN EXPOSURE

Total dose of 2,4-D found in 96 hour urine samples supplied by volunteers is presented in Tables 10 and 11. Creatinine values were calculated in mmoles from daily volume measurements for four 24 hour periods for each volunteer and a mean of the 4 days is reported. All creatinine values were above or fell within the normal range for adult females (5.0 - 16.0

mmole/24 hours) and for adult males (7.0 - 18.0 mmole/24 hours), and showed little variation on a daily basis, indicating complete sample collection.

Table 10 Total dose of 2,4-D found in urine samples supplied by volunteers exposed to turf 1 hour following a 2,4-D application.

Volunteer	Weight (kg)	Creatinine mmole/day	Spiked recovery	Baseline exposure	Total μg 2,4-D	2,4-D $\mu\text{g}/\text{kg}$
1 shorts	100.0	19.86	76.01	ND ¹	153.06	1.53
2 shorts	95.5	19.87	84.36	ND	ND	0.00
3 shorts	63.6	11.37	89.48	ND	ND	0.00
4 shorts	45.5	7.12	113.4	ND	103.06	2.27
5 shorts	79.5	18.00	97.54	ND	426.44	5.36
6 pants	77.3	14.84	97.92	ND	ND	0.00
7 pants	68.2	9.83	81.82	ND	ND	0.00
8 pants	72.7	12.29	99.90	ND	ND	0.00
9 pants	65.9	14.67	76.29	6.58	**	**
10 pants	79.5	15.20	lost	ND	ND	0.00

¹ Non-detectable < 5 $\mu\text{g}/\text{L}$

**removed from study due to positive baseline exposure

The highest exposure encountered in a volunteer (#5, shorts) occurred 1 hour following application. Exposure of this volunteer was at least two times higher than other volunteers wearing shorts when considering both total dose and dose on a mg/kg body weight basis. No 2,4-D was detected in urine samples of volunteers wearing long pants and closed footwear (Table 10) or in either group 24 hours following application (Table 11). Two volunteers were removed from the study due to positive baseline exposures. While the area of skin exposed to treated surfaces can affect total dose absorbed, this does not account for variations seen within the 1 hour exposure group wearing shorts. As with all human experiments, the individual variation between subjects is the most difficult to control. The absorption of pesticides through human skin occurs at different rates and personal habits (i.e. how people sit or lie) may affect either area of contact with the treated surface or the anatomic site of the body exposed. The time before bathing or showering will affect the absorption of the pesticide and various other factors such as amount of exercise and sweating, and the wearing of contaminated clothing, may affect absorption.

The relationship between total dose and dislodgeable residues of 2,4-D is shown in Table 12. Average total dose for the 1 hour re-entry period was calculated from volunteer #'s 1 and

4 total dose measurements, assuming a worst case scenario but, for sake of comparison with the 24 hour re-entry period, volunteer # 5 was not included. Volunteer 5 was not included because he removed his shirt for part of the exposure session and was therefore not representative of the other members in the group. An absorption factor was calculated by dividing the amount of 2,4-D (mg) available for human contact by the average of the two exposures. This factor was used to estimate the theoretical total dose of 2,4-D which could be found in urine samples of volunteers in the 24 hour re-entry exposure session. This dose was calculated at 16.8 µg. This is the theoretical amount of 2,4-D that would be recovered in a volunteer who wore shorts and showed no variation from the two volunteers in the 1 hour exposure session. All five volunteers had urine volumes of at least 4 litres, over the 4 day period, which would reduce exposure to non-detectable levels (5 ppb). It is thus possible that these people were in fact exposed to 2,4-D, but below the limit of detection.

Table 11 Total dose of 2,4-D found in urine samples supplied by volunteers exposed to turf 24 hours following a 2,4-D application.

Volunteer	Weight (kg)	Creatinine mmole/day	Spiked recovery	Baseline exposure	Total µg 2,4-D	2,4-D µg/kg
1 shorts	100.0	18.83	81.79	ND ¹	ND	0.00
2 shorts	77.3	13.5	103.51	ND	ND	0.00
3 shorts	63.6	10.66	79.41	ND	ND	0.00
4 shorts	79.5	11.86	95.02	ND	ND	0.00
5 shorts	72.7	11.78	84.43	ND	ND	0.00
6 pants	75.0	13.94	121.40	ND	ND	0.00
7 pants	63.6	8.42	88.26	22.82	**	**
8 pants	67.3	11.20	84.49	ND	ND	0.00
9 pants	65.9	14.04	73.5	ND	ND	0.00
10 pants	100.0	15.03	113.89	ND	ND	0.00

¹ Non-detectable < 5 µg/L

** removed from study because of positive baseline.

Table 12 The relationship between dislodgeable residues and total dose of 2,4-D calculated in 2 volunteers.			
		1 Hour	24 Hours
Amount of 2,4-D applied in each plot (g):		3.3	3.3
Percent dislodgeable:		7.6	1.0
Amount of 2,4-D available for human contact (mg):		251	33.0
Average total dose (μg):		128 ¹	16.8 ²
Absorption Factor:		1961	1961

1 Average of volunteer #'s 1 and 4.

2 Theoretical dose estimated by dividing amount available for human contact with absorption factor.

In summary, it appeared that type of clothing worn during exposure to sprayed turf affected the total dose of 2,4-D absorbed in humans. In addition, as dislodgeable residues declined, human exposure to 2,4-D decreased. The highest total dose calculated in any volunteer was less than 0.5 mg. This is much lower than the acceptable daily intake of 23.9 mg (0.3 mg/kg/day) suggested by the World Health Organization (WHO, 1984). However, people who wish to reduce exposure to non-detectable levels, can remain off treated turf for a period of 24 hours or until after rainfall or irrigation so that dislodgeable residues and therefore potential exposure are essentially zero.

It must also be considered that children may be exposed to sprayed turf by routes uncommon to adults. Adult exposure is mainly dermal, while oral and inhalation exposure are minimal for 2,4-D. Children may eat grass or dirt and ingest the pesticide. This should be taken into consideration when advising the public on exposure reduction practices.

3.4 PERCUTANEOUS PENETRATION OF 2,4-D ACID AND 2,4-D DMA IN HUMAN VOLUNTEERS

The amount of 2,4-D detected in hand wash and urine samples is presented in Table 13 (and in graphical form in Appendix D). Volunteers excreted an average of 4.43 ± 0.847 % of the applied acid and 1.76 ± 0.568 % of the applied DMA salt. To test the null hypothesis that the two formulations would result in the same absorption, the data was analyzed as a paired set and tested for significance at the 2.5% level with a two-tailed Student's t-test. The null hypothesis was rejected and therefore, significantly higher absorption of the acid was found in volunteers. The same analysis of hand wash data showed that a significantly higher (2.5%) of DMA salt was washed off the back of the hand than the applied 2,4-D acid. These results suggested that a relationship existed between the formulation of 2,4-D and the percent of dose absorbed. It also appeared that absorption was inversely related to the amount of chemical that was washed off the skin. It was not possible to account for the total dose of 10 mg applied when urinary excretion values and handwash results were combined. Pesticide may have been

dislodged from the skin by physical rubbing although all volunteers were careful not to disturb the area. Other routes of excretion, such as through perspiration and faeces were not determined. In addition, it is thought that the skin can act as a reservoir and only small amounts of the chemical are released into the bloodstream and subsequently processed by the kidneys and excreted in the urine. Post-exposure release of the pesticide residues "stored" in the skin may be slow enough to keep concentrations below the limit of detection in urine.

Many factors will affect the percutaneous absorption of a pesticide. The concentration of the applied dose, and surface area and site of the application have been shown to be major factors affecting absorption. These factors were held constant in these experiments. The chemical nature of the pesticide may also affect absorption. The hydrated *stratum corneum* has affinity for both water soluble and lipid soluble non-electrolytes. However, as the polar character of a penetrant molecule is increased, lower permeability has been reported (Scheuplein, 1978). 2,4-D DMA is infinitely soluble in water whereas 2,4-D acid is only slightly soluble (52.2 mg/100 mL at 25°C, QueHee and Sutherland, 1981). This difference in solubility may result in decreased absorption of the DMA formulation as compared to the acid.

Volunteer:	1	2	3	4	5	Mean ± S.D.
2,4-D acid recovered (µg/6 days) ¹ :	327.1	709.7	487.3	209.2	501.3	
2,4-D as a % of applied:	3.27	7.09	4.87	2.09	5.01	4.43 ± 0.847
2,4-D acid in handwash (mg) ² :	6.60	5.16	4.54	4.64	5.79	5.35 ± 0.384
Amount unaccounted for (mg):	3.07	4.13	4.97	5.15	3.71	
2,4-D DMA recovered (µg/6 days) ¹ :	108.6	328.0	5.95	173.4	266.3	
2,4-D as a % of applied:	1.09	3.28	0.0595	1.73	2.66	1.76 ± 0.568
2,4-D DMA in handwash (mg) ² :	8.28	7.32	9.22	7.22	6.34	7.68 ± 0.493
Amount unaccounted for (mg):	1.61	2.35	0.77	2.61	3.39	

1 Corrected for spiked urine recovery.

2 Corrected for spiked water recovery.

The vehicle used to dissolve the chemical for application to skin may also affect absorption. Percutaneous absorption of a chemical from a vehicle depends on the partitioning of the chemical between the vehicle and the skin and the solubility of the chemical in the vehicle. In addition, the vehicle may change the integrity of the skin and influence absorption (Webster and Maibach,

1983). Various non-aqueous solvents such as alcohols, dimethylsulfoxide (DMSO) and benzene have been tested to determine the effect on absorption. These solvents and chemicals can act as membrane conditioners and affect absorption by producing changes in the physical parameters or the chemical nature of the membrane. Thus, the distribution coefficient between the applied product and the skin may be altered. When the integrity of the *stratum corneum* is disturbed, it commonly results in increased penetration of chemicals (Scheuplein, 1978; Wurster, 1978).

Feldman and Maibach (1974), found that $5.8 \pm 2.4\%$ of the applied dose of 2,4-D acid to the ventral forearm could be recovered in the urine in 120 hours. The area of skin was not protected and was washed 24 hours following the application. The time period may have allowed for greater absorption of the compound as compared to results reported in this paper. It is also known that a regional variation in percutaneous penetration is seen at different anatomic sites (Maibach *et al.*, 1971). Absorption of parathion, malathion and carbaryl was two times higher when applied to the dorsum of the hand as compared to the ventral forearm. If it is assumed that 2,4-D shows a similar regional variation in absorption, the results reported for the hand dorsum should approach approximately 10% absorption for the 2,4-D acid.

The percutaneous absorption of 2,4-D dimethylamine salt was significantly lower than 2,4-D acid. Other pesticides may show the same pattern. It should be considered that, to calculate total body dose from dermal exposure studies, the typical absorption factor of 100% is as unrealistic as it is deceptive.

The amount of 2,4-D excreted in the urine in 96 hours following a dermal application as a percentage of total recovered dose for both 2,4-D acid and 2,4-D DMA is presented in Table 14. Excretion was not complete following the 2,4-D acid application in 3 of the 5 volunteers, and following the 2,4-D DMA application in 1 of the 5 volunteers. In all cases the levels detected in the final 24 hours of the study were approaching the limit of detection (5 ppb). Therefore, for ease of calculation, it was assumed that 100% of the absorbed dose was excreted in the 144 hour period. Similar results have been obtained by Kohli *et al.*, (1974), who found that $76.5 \pm 8.4\%$ was excreted in the urine in 96 hours following a single oral dose (5 mg/kg) to 6 human subjects. Many 2,4-D exposure studies have made use of a four day collection period, following one exposure, to determine total absorbed dose. Results indicate that, particularly with 2,4-D DMA, this 4 day collection period may not be adequate for all volunteers. However, due to the great variation in excretion rates, it is not possible to average the 96 hour values for each volunteer and correct for incomplete recovery.

Half-lives for urinary elimination were calculated assuming a linear excretion over time and are presented in Table 14. Results of previous studies (Feldman and Maibach, 1974) have shown that the half-life for urinary elimination of 2,4-D following an intravenous administration is 13 hours. Following oral administration, average half-lives for clearance from urine have been calculated at 17.7 hours (Sauerhoff *et al.*, 1977) and from plasma at 33.0 ± 3.1 hours (Kohli *et al.*, 1974). Obviously, a great deal of variation occurs in human subjects. One would suspect that the half-life for excretion of 2,4-D following dermal administration should be longer than those following intravenous or oral administration partially due to the fact that the compound must penetrate through the skin into the bloodstream.

Table 14 Percent of total 2,4-D excreted in urine in 96 hours and approximate half-life of urinary excretion following dermal application of 2,4-D acid and 2,4-D DMA in 5 male volunteers.						
Volunteer	1	2	3	4	5	Mean \pm S.E.
2,4-D acid application						
% Total dose excreted in 96 hours	89.6 ¹	87.9	83.1 ¹	75.6 ¹	87.9 ¹	84.8 \pm 2.55
Half-life (hours)	18.0	33.5	38.5	68.0	39.5	39.5 \pm 8.10
2,4-D DMA application						
% Total dose excreted in 96 hours	66.9	84.5	100	52.5	79.9 ¹	76.8 \pm 8.05
Half-life (hours)	87.1	37.2	18.0	79.2	70.9	58.5 \pm 13.2

¹ Excretion of 2,4-D was not complete at 144 hours but approaching the limit of detection.

4 THE USE OF 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D) EXPOSURE DATA IN HUMAN RISK ASSESSMENT

4.1 INTRODUCTION

Broadly defined, risk assessment is the qualitative or quantitative estimation of the likelihood of adverse effects from exposure to specified health hazards or from the absence of beneficial influences. The process of risk assessment is typically composed of four steps: hazard identification, exposure assessment, dose-response assessment and risk characterization or determination. Not all four of these steps may be necessary in every situation and therefore only dose-response assessment will be discussed.

4.2 DOSE-RESPONSE ASSESSMENT

Dose-response assessment is concerned with the evaluation and quantitative characterization of the relationship between the level of exposure to a pesticide and the toxicologic response, particularly as it applies to humans. Three techniques frequently used in this process will be described. Total body dose calculations from recent 2,4-D applicator exposure studies are presented in Table 15. Results of only biological monitoring studies are presented because most dermal exposure studies have assumed 100% absorption of 2,4-D through the skin and have therefore lead to gross over-estimations of total dose received in applicators.

4.2.1 SAFETY FACTOR

The safety-factor approach is the most common technique used for estimating risk when the endpoints are noncarcinogenic. It is used to determine an "acceptable" or "allowable" human intake level of a pesticide often referred to as the Acceptable Daily Intake (ADI) (Franklin, 1985; Stevens and Sumner, 1985). This approach is commonly used for pesticides which exert toxicological effects that demonstrate a no-observed-effect-level (NOEL). The NOEL is determined by animal toxicity testing and then divided by an "appropriate" safety factor to allow for errors in extrapolation of animal data to humans and variations in human sensitivity.

In a long-term feeding study of 2,4-D in rats, a NOEL was equivalent to 31 mg/kg/day (WHO, 1984). Based on this, an ADI of 0.3 mg/kg/day for humans has been established. It should be noted that the NOEL for all of the chronic adverse effects of 2,4-D in mammals has not been firmly established, and quite recently the U.S. Environmental Protection Agency (EPA) has defined a NOEL in rats of 1 mg/kg/day based on long-term feeding studies conducted by the Hazleton Laboratories (1986) under the supervision of the Industry Task Force on 2,4-D. It is possible that the ADI for humans will be re-evaluated. However, the safety-factor approach is simple to apply and is widely used when interpreting results of 2,4-D exposure studies. Table 16 shows the fraction of the ADI for each exposure study listed in Table 15. An average body weight of 70 kg was used to compute the daily and lifetime exposures for each group on a mg/kg body weight basis. For simplicity, total lifetime exposure calculations used the values of a 365 day year and a 70 year lifetime. This approach has been questioned as it results in a very small estimated daily exposure and ignores the toxicological significance of high pulses of exposure. There are generally no toxicology data to support this approach (Franklin, 1985) but it should be noted that not one exposure to 2,4-D encountered in these studies exceeded the ADI on any one day.

4.2.2 MARGIN OF SAFETY

The margin-of-safety (MOS) procedure is closely related to the safety-factor approach and is obtained by dividing the NOEL by the estimated human exposure level.

$$\text{MOS} = \text{NOEL (mg/kg/day)} / \text{Exposure (mg/kg/day)}$$

The more recently defined NOEL of 1 mg/kg/day in rats has been used for these calculations with results presented in Table 16.

Table 15 The occupational exposure of persons applying 2,4-D in relation to application method.

Method of 2,4-D application	Occupation	Dose mg/day	Days/year exposed	Years exposed	Total intake (mg)
<i>Libich (1981) and Libich et al. (1984)</i>					
Packsprayer-handheld gun	ROW ¹ sprayer	3.53	60	10	2107.5
	roadside sprayer	3.45	60	10	2070.0
Mist blower	ROW sprayer	4.86	60	10	2895.0
<i>Frank et al. (1985)</i>					
Helicopter	mixer-loader	1.043	12	20	250.3
	mixer-flagger	0.465	12	20	111.6
	flagger	0.336	12	20	80.6
Airplane	mixer-loader	0.153	12	20	36.7
	supervisor	0.005	12	20	1.2
<i>Grover et al. (1986)</i>					
Tractor	farmer	0.475	14	25	166.2
<i>Yeary, (1986)</i>					
Hand-held gun	commercial sprayer	0.29	66	13	245.4
<i>Harris et al. (1990)</i>					
Hand-held wand	commercial sprayer	0.26 ²	66	13	223.1
		1.1 ³	66	13	943.8
	mixer/loader	0.16	66	13	137.3
Hose-end sprayer	Homeowner applicator	0.744 ⁴	2	20	29.8

1 Right-of-way sprayer

2 Based on the average exposure of all professional applicators

3 Based on the highest exposure determined in any applicator

4 Based on the highest exposure determined in a homeowner applying liquid 2,4-D with no protective clothing.

Many problems are associated with both the safety-factor approach and the MOS. The calculated NOEL is highly dependent on the sample size and the sensitivity of the test species and these approaches do not account for the shape or slope of the dose-response curve for a particular response of interest. These methods imply the existence of an actual or practical threshold, a condition which is difficult to justify biologically or calculate empirically (Franklin, 1985; Munro and Krewski, 1981). Finally, the interpretation of the results is difficult and is subject to personal judgement. What is considered an acceptable risk for one individual may be considered an unnecessary risk for another.

4.2.3 CONCLUSIONS

Most risks associated with occupational or bystander exposure to 2,4-D are low, especially when compared to other risks taken in life. However, public perception regarding 2,4-D and for that matter, pesticides in general, is extremely negative, despite the low values of these calculated risks.

Table 16 Occupational exposure expressed as the margin of safety and as a fraction of the ADI on a daily and life time basis.

Method of 2,4-D application	Occupation	Average intake (mg/kg/day)	Fraction of ADI (1 day)	Fraction of ADI (lifetime)	MOS ¹
Libich (1981) and Libich <i>et al.</i> (1984)					
Packsprayer-handheld gun	ROW ²	0.05	1/6	1/255	20
	roadside sprayer	0.05	1/6	1/259	20
Mist blower	ROW sprayer	0.07	1/4	1/185	14
Frank <i>et al.</i> (1985)					
Helicopter	mixer-loader	0.01	1/30	1/2143	100
	mixer-flagger	0.007	1/43	1/4808	143
	flagger	0.005	1/60	1/6654	200
Airplane	mixer-loader	0.002	1/150	1/14612	500
	supervisor	0.00007	1/4286	1/447125	14286
Grover <i>et al.</i> (1986)					
Tractor	farmer	0.007	1/43	1/3228	143
Yeary, (1986)					
Hand-held gun	commercial sprayer	0.004	1/75	1/2187	250
Harris <i>et al.</i> (1990)					
Hand-held wand	commercial sprayer	0.004 ³	1/75	1/2405	250
		0.02 ⁴	1/15	1/601	50
	mixer/loader	0.002	1/150	1/3908	500
Hose-end sprayer	Homeowner applicator	0.007 ⁵	1/43	1/18029	143

1 Margin of Safety for a one day exposure

2 Right-of-way sprayer

3 Based on the average exposure of all professional applicators

4 Based on the highest exposure determined in any professional applicator

5 Based on the highest exposure determined in a homeowner applying liquid 2,4-D with no protective clothing (100 kg).

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