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16. Abstract

The present study was conducted for four consecutive years to assess the exposure of field workers applying herbicide mixtures (2,4-D + Roundup and Garlon 3A + Roundup) and to determine the toxicity and fate of these herbicides in the environment. Study results indicated that exposure of workers to these mixtures via inhalation was insignificant compared to threshold limit values (TLVs) set by the American Conference of Governmental Industrial Hygienists (ACGIH, the National Institute for Occupational Safety and Health (NIOSH) and the Occupational Safety and Health Administration (OSHA) for the protection of human health. In addition, herbicide concentrations in the urine of these workers were much higher than those found in air samples collected from breathing zones. This was probably due to exposure via ingestion (eating without proper hand washing) and/or exposure via dermal contact. The analysis of samples taken from the environment including sediment and water collected over three years indicated that 2,4-D, Roundup and Garlon-3A did not pose an immediate (acute) risk to aquatic life in water bodies adjacent to herbicide treated areas on the right-of-way. Moreover, fish, crawfish and microbial acute bioassay results showed that harmful concentrations were not likely to occur as a result of herbicide use under current application rates recommended by the manufacturers.

Recommendations For Further Assessment

(1) Assess the potential exposure of Mobile Equipment Operators, MEO's (open mowing tractors). They could be exposed via inhalation and/or dermal contact by contaminated grass and dust particles. (2) Study the extent and impact of herbicides' drift. (3) Evaluate subchronic and chronic effects of these herbicides on freshwater organisms. (4) Investigate the bioavailability of these compounds to life forms lining in treated areas. (5) Study the fate of grass particles resulting from immediate mowing following spraying. Rains and winds may carry contaminated cut grass to adjacent water bodies causing a concern of water contamination.

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ASSESSMENT OF THE EXPOSURE OF WORKERS APPLYING HERBICIDE MIXTURES (2,4-D+ROUNDUP, GARLON-3A+ROUNDUP), TOXICITY AND FATE OF THESE MIXTURES IN THE ENVIRONMENT

SUMMARY REPORT

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ABSTRACT

The present study was conducted for four consecutive years to assess the exposure of field workers applying herbicide mixtures (2,4-D + Roundup and Garlon 3A + Roundup) and to determine the toxicity and fate of these herbicides in the environment. Study results indicated that exposure of workers to these mixtures via inhalation was insignificant compared to threshold limit values TLVs) set by the American Conference of Governmental Industrial Hygienists (ACGIH), the National Institute for Occupational Safety and Health (NIOSH) and the Occupational Safety and Health Administration (OSHA) for the protection of human health. In addition, herbicide concentrations in the urine of these workers were much higher than those found in air samples collected from breathing zones. This was probably due to exposure via ingestion (eating without proper hand washing) and/or exposure via dermal contact. The analysis of environmental samples including sediment and water collected over three years indicated that 2,4-D, Roundup and Garlon-3A did not pose an immediate (acute) risk to aquatic life in water bodies adjacent to herbicide treated areas on the right-of-way. Moreover, fish, crawfish and microbial acute bioassay results showed that harmful concentrations were not likely to occur as a result of herbicide use under current application rates recommended by the manufacturers.

IMPLEMENTATION STATEMENT

While low levels of herbicides were detected in samples collected from humans and the environment, care should be taken in their application.

1) In this study, low levels of 2,4-D and Garlon 3A (Triclopyr) were detected in the air collected from breathing zones. Although the levels were much below the ACGIH recommended threshold limit values (TLVs), precautions should be taken. All herbicide applicators should stay inside the cab and windows should be kept closed during spraying.

2) Herbicide levels were much higher in urine than in air. This means that herbicides entered the body from other than inhalation, possibly through ingestion or absorption through the skin. Hands must be properly washed after handling the herbicides especially before meals. Protective clothing should be worn and spills on skin must be washed with water.

3) The trend of levels of herbicides in the urine of workers during four spray seasons indicated a slight increase during each season. However,urine results showed no carry over from one spray season to another.

4) To avoid aerosol inhalation,it is advisable to stand upwind of the prevailing wind direction when mixing the herbicides. Spraying rigs should be modified (if and when possible) so nozzles are located at the back of the truck.

5) Results of testing herbicide mixtures on fish and crawfish indicate low acute toxicity values; however, this does not preclude toxicity to other aquatic species. Chronic toxicity to all aquatic life forms may also be of concern. Therefore, spills and direct application of herbicides to water should be avoided.

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INTRODUCTION

It is generally accepted that mixtures of chemicals are more effective than chemicals applied individually. This is becoming increasingly apparent with the use of pesticides for agricultural and other environmental applications. Mixtures often show greater effectiveness against target organisms, with less toxic effects on nontarget organisms. Of equal importance, mixtures can often be applied at rates below the levels required for single compounds, thereby reducing total chemical application, residuals in the environment and cost.

The application of mixtures, however, is not without disadvantages. Some chemical mixtures can pose a greater danger to nontarget species and to the environment than the individual compounds. Only by understanding the additive toxicity caused by chemicals in combination, can the potential effects of particular chemical mixtures be evaluated.

The State of Louisiana Department of Transportation and Development (LADOTD) uses a mixture of two herbicides, the isopropyl amine salt of 2,4-D and Roundup containing the active ingredient glyphosate, to control weed growth along the state's highways. A third herbicide, Garlon 3A, which contains the active ingredient Triclopyr has recently been added to the program to replace 2,4-D in certain parts of the state. These herbicides are used on roadsides to kill weeds and thereby extend the life of the pavement, prevent obstruction of the signs, maintain sight distance, and to provide a neat grass covered roadway not subject to erosion or fire.

While data on the toxic effects of these herbicides as individual compounds are available in the literature, it is important to address their behavior as chemical mixtures to assess potential interactions which may increase or decrease their impact on man and his environment.

The purpose of this study was to assess the acute toxicity, fate and environmental impact of herbicide mixtures, including Roundup/Garlon-3A and Roundup/2,4-D. Such a study is of significance to the LADOTD that uses these chemical mixtures for the control of weed and woody plants along roadside rights-of-way in Louisiana.

STUDY OBJECTIVES

The objectives of this study were:

1. To assess chemical exposure of workers applying 2,4-D, Garlon-3A, and Roundup in three field locations in Louisiana, through monitoring herbicide levels in the urine of herbicide workers and in air samples collected from breathing zones.

2. To determine the acute toxicity of Roundup, Garlon-3A, 2,4-D and their surfactant, (both singly and as herbicide mixtures) to catfish, bluegill sunfish, crawfish and soil microorganisms to evaluate potential antagonistic, additive or synergistic effects.

3. To determine the levels of two mixtures (Roundup/Garlon-3A and Roundup + 2,4-D) in soil, grass and water collected from areas sprayed with the herbicide mixtures.

4. To examine the sorption capacity of Louisiana soils to herbicide mixtures.

5. To study the fate of herbicide mixtures in microcosms.

LITERATURE REVIEW

Herbicides are chemicals which are able to interfere with the basic biochemical photo processes and in doing so, disturb vital functions in plants (Sitting, 1971). Herbicides vary in their nature and mode of action. They have many applications in agriculture where they are widely used for weed control. They may affect either or both dicotyledonous (broad leaf) plants, monocotyledonous (grasses) plants. They can be absorbed through the roots or the leaves of the plants. Those which affect the plant at the site of contact are called contact herbicides and those which the site of action is different than the site of contact are called systemic herbicides (Aldus, 1976).

Although the literature is scarce on the fate and toxicity of Triclopyr and glyphosate in the environment, extensive studies have been carried out with the 2,4-D herbicide. In 1987, Lavy and his research group at the University of Arkansas studied a variety of herbicides and application methods. Results of their studies indicated that a very small portion of the applied dose was received by inhalation, and 99% of the dose came through the skin. The same researchers (1982) conducted a study with forestry helicopter workers spraying 2,4-D and found that the levels of 2,4-D in the air samples were below detection limit, while the levels on dermal patches ranged from below detection limit (0.037 mg) to 2.974 mg on a headband patch. In urine samples, 2,4-D ranged from below detection limit to 0.04 ppm. In 1983, Kolmodin-Hedman and collaborators found that after exposure to 2,4-D, workers had a total of 2 to 5 ppm of 2,4-D in urine samples. The half life ($T_{1/2}$) was 12 hours. In 1986, Yeary reported a urine concentration of 2.5 ppm for workers operating car mounted rigs (Knopp and Glass, 1991). In a study on human exposure to 2,4-D, Harris and Solomon (1992) concluded that exposure to sprayed turf should present little risk in humans.

In 1979 and 1980, workers applying herbicides to power line rights-of-way in Canada were studied. A 2,4-D/picloram mixture was applied from either vehicles or by backpack sprayers. The study examined dosages received according to application method. Between the two study years, improvements in safety procedures were instituted and wash-up facilities were provided which resulted in a decrease in exposed doses. Air samples in 1980 for the vehicular and backpack sprayers contained 2,4-D mean concentrations of 7.1 to 13.5 ug/m^3 and 55.2 ^{3}ug/m , respectively. The picloram concentrations were 1.3 to 2.3 ug/m^3 and 14.9 ug/m^3 for vehicular and backpack sprayers, respectively (Libich et al., 1984).

Lavy et al. (1987) studied the urine of forest workers applying Tordon 101-R, a manufactured mixture of 80% 2,4-D and 20% picloram. The herbicide 2,4-D ranged from 0.04 to 0.24 mg 2,4-D /kg body weight while picloram was found at below detection levels to 0.02 mg picloram /kg body weight. Assuming that picloram and

2,4-D were equally deposited in the 4:1 ratio, a smaller portion of the picloram was absorbed through the skin than 2,4-D.

The National Health and Nutrition Examination Survey II examined background levels of many pollutants in the U. S. population. One percent of the population had been found to have 2,4-D present at concentrations greater than 30 ppb (Murphy et al., 1983).

Field studies of the persistence of a 2,4-D and glyphosate mixture were performed by Torstensson et al. (1989) in northern Sweden. Results of these studies indicated that the half-lives of both chemicals were less than 45 days. Also, these compounds tended to persist longer in areas where microbial activity was low due to freezing weather. In a study evaluating the effects of soil factors on the degradation of herbicides, Schoen and Winterlin (1987) found that the degradation of 2,4-D was most affected by the organic content of soil, pH, microbial population and herbicide concentration.

The United States Environmental Protection Agency (USEPA) has designated an official method for 2,4-D, glyphosate and Triclopyr analysis in environmental samples. These methods are described in SW-846, Test Methods for Evaluating Solid Waste (USEPA, 1983, 1986, 1994; NIOSH, 1984). Standard Methods for the Examination of Water and Wastewater (APHA), 1992) also contains a method for 2,4-D analysis. These methods address both soil and environmental (water) samples. A third method exists which is used for the analysis of 2,4-D in drinking water. The official method is adapted to a matrix which is much cleaner and expected to contain only trace quantities of the herbicides with minimal interference from other organic acids.

MATERIALS AND METHODS

A. HUMAN EXPOSURE STUDIES

1. Urine

A half gallon plastic jug with a 'polyseal' cap was supplied to each worker every day. The jug was labelled with name and date by the worker. The participants were required to carry the container with them all day. Samples collected from these participants by Tulane personnel were transported on ice to the laboratory. All samples were analyzed for test herbicides. Triclopyr and 2,4-D were analyzed by Gas chromatography while glyphosate was assessed by High Performance Liquid Chromatography.

2. Air

Air sampling was done during an entire spray season. Thirteen workers operating spray equipment were recruited as human subjects for herbicide deposition. Four workers were obtained from Baton Rouge (2,4-D and glyphosate), three from Hammond (Triclopyr and glyphosate) and six from the Bridge City crew (2,4-D and glyphosate).

Model 224-43XR air sampling pumps, were purchased from SKC, Eighty-four, Pennsylvania. They were first calibrated in the laboratory using an SKC Accuflow Digital Calibrator.

During the spraying season, pumps were calibrated in the field at least once a week with the SKC automated calibrator at least once a week to ascertain a flow of 0.37 liters per minute.

B. TOXICITY TESTING

Four chemicals (three herbicides and the surfactant) were used in this study: Garlon 3A (Triclopyr), 2,4-D or Weedestroy AM-40, Roundup (glyphosate), and Syndets anionic surfactant. All chemicals were of agri-chemical grade.

Channel catfish (*Ictalurus punctatus*, Rafinesque), bluegill sunfish (*Lepomis macrochirus*), crawfish (*Procambarus spp*) and a heterogenous population of microorganisms derived from soil were used as test organisms.

Fish and crawfish bioassays (96-hrs static renewal) were conducted following standard testing protocols (USEPA, 1985; APHA, 1992). In testing with chemical mixtures, the procedure described by Marking was applied (Marking, 1985). Specific microbial assays including the growth inhibition test (Alsop et al., 1980), the oxygen depletion test (Anderson et al., 1988) and the Microtox (Ribo and Kaiser, 1987) were used in studying the effects of test compounds on bacteria.

C. SOIL TESTING

Adsorption and desorption studies were conducted on soils obtained from the control areas adjacent to samples sites on the US 61 near Baton Rouge, I-55 at Hammond and US 90 at Bridge City (Highway 90).

Soil characterization tests were performed by the LADOTD Soils Laboratory in Baton Rouge. Soil composition was determined by sieve analysis, using a #40 and #200 sieve, to ascertain the content of sand, silt and clay. A hydrometer analysis was conducted to delineate the nature and composition of the clays in the soil. The Atterberg Plastic and Liquid Limits tests were performed to further characterize soil type and compressibility. The organic content of the soil was determined by the Wet Oxidation-Redox Titration Method (Tiessen and Moir in Carter, 1993.)

D. MICROCOSM STUDIES

Blocks of soil and grass, ranging from 0.5 to 1 cubic foot, were dug from sampling sites on the highways near Baton Rouge (US 61), Hammond (I-55) and Bridge City (US 90) and transported undisturbed to the laboratory where they were placed in 40 gallon aquaria so that one half to two thirds of the tank was soil and grass and the other portion aqueous. Sediment (1 gallon) was also collected at each site.

Tanks were sprayed with field application solutions of 2,4-D, Triclopyr and Round-up. Rainfall was applied to tanks based on the average of rainfall in the last ten years in Louisiana. Soil, grass, water and sediment samples were collected at different intervals and analyzed for glyphosate and either 2,4-D or Triclopyr.

E. ENVIRONMENTAL FIELD SAMPLES

Environmental samples including grass, soil at 0 and 3" (0-7.5 cm), water and sediment were collected from three selected sites (Hammond, Baton Rouge and Bridge City) in the state. Samples were collected quarterly during 1990, 1991, and 1992. All samples were analyzed for the test herbicides.

RESULTS AND DISCUSSION

A. HUMAN EXPOSURE MONITORING

Three applicator crews (gangs) were studied during the spray seasons of 1990, 1991, 1992 and 1993. These included workers in Baton Rouge, Bridge City and Hammond. A fourth group from Chase was added to the study in 1993. Mixtures of 2,4-D and Roundup were applied in Baton Rouge and Bridge City, while mixtures of Triclopyr and Roundup were applied in Hammond and Chase. In addition, 2,4-D was also applied in Chase.

Exposure of workers to herbicides was measured during the spray seasons of 1990 to 1993. The amounts of herbicides from the breathing zone during the work day and 24-hour urine composites were sampled.

Figures 1, 2, 3 and 4 illustrate the trends in 2,4-D levels (between 1990 and 1993) in the urine of herbicide applicators working at Baton Rouge (participants 1, 2, 3 and 4). Similar illustrations are presented in Figures 5, 6 and 7 for the Triclopyr levels in urine of Hammond workers (participants 5, 6 and 7). For those working in Bridge City, the trends of 2,4-D levels in their urine from 1990 to 1993 are depicted in Figure 8 (Participant 8), Figure 9 (participant 9), Figure 10 (participant 10) and Figure 11 (participant 11).

Fig 2. Trend of 2,4-D Levels in Urine Worker 2 During Four Spray Seasons

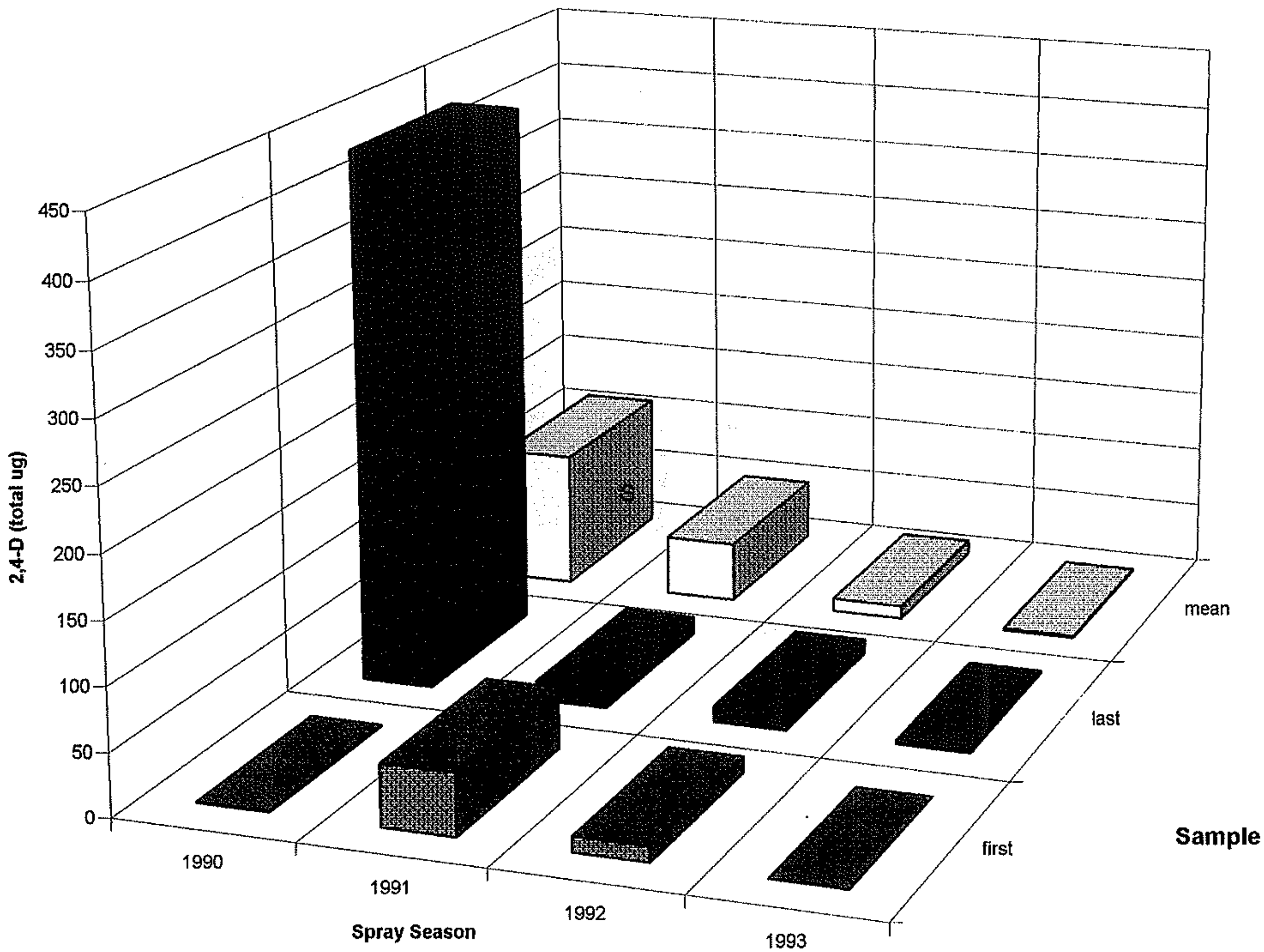


Fig 3. Trend of 2,4-D Levels in Urine of Worker 3
During Four Spray Seasons

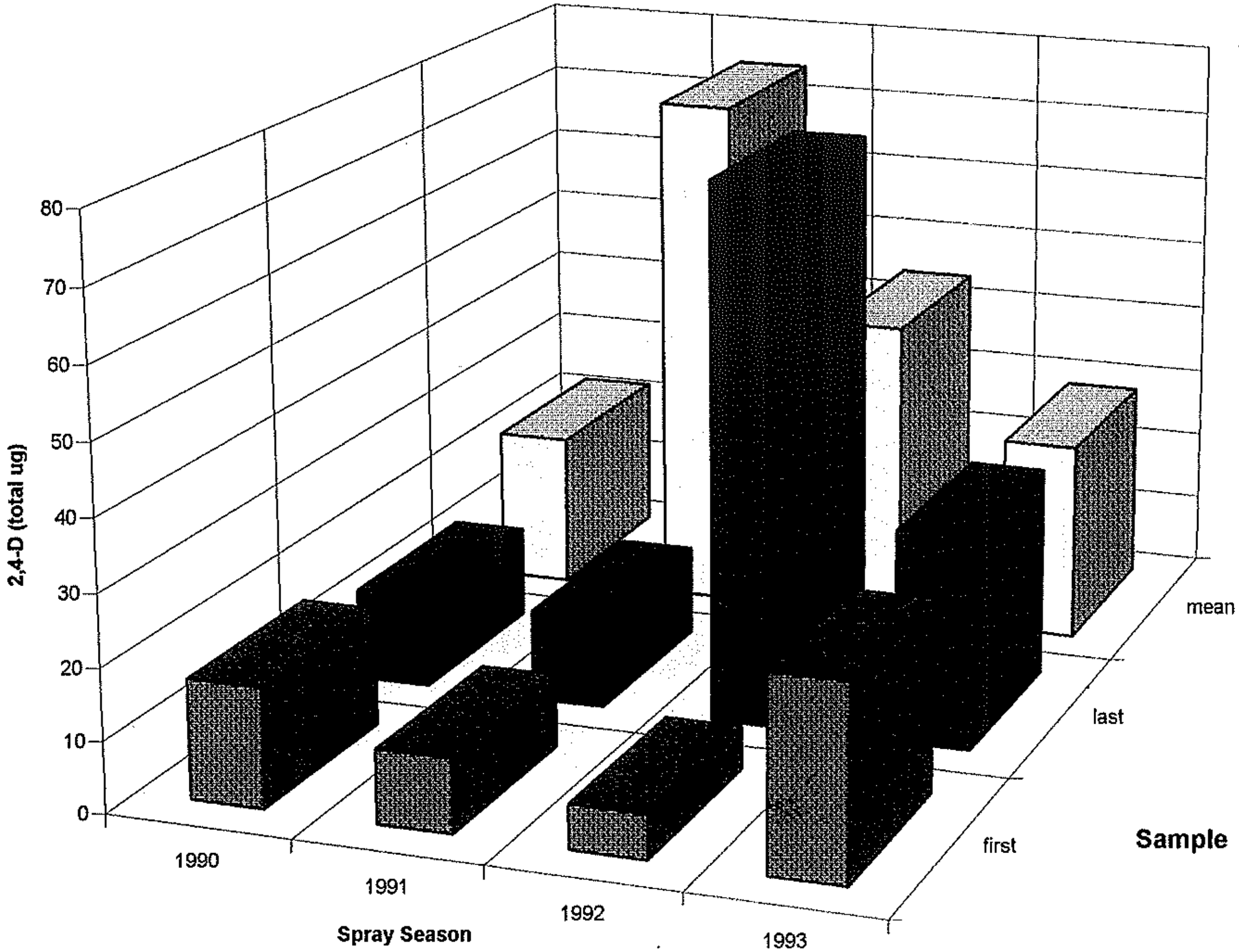


Fig 8. Trend of 2,4-D Levels in Urine of Worker 8
During Four Spray Seasons

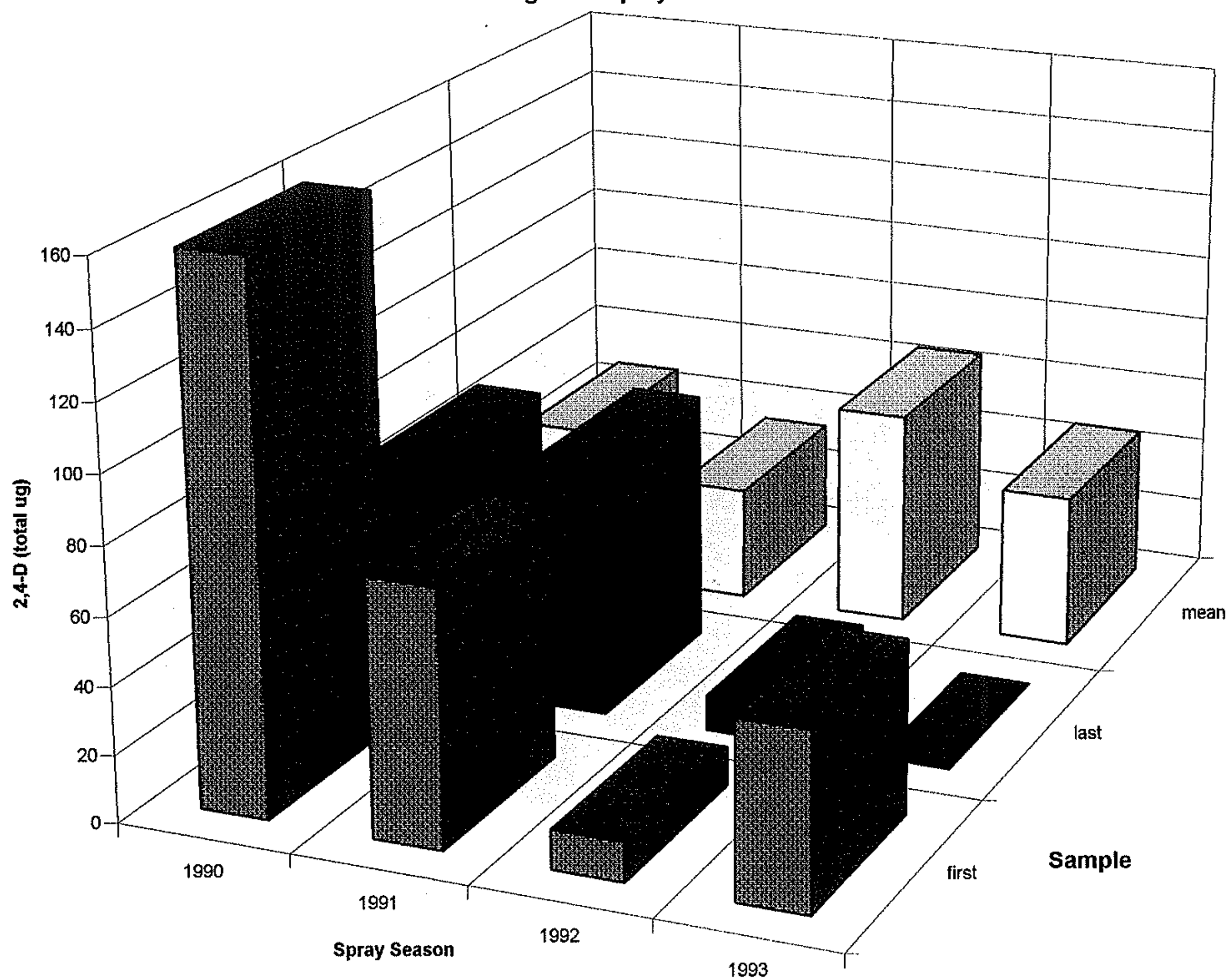
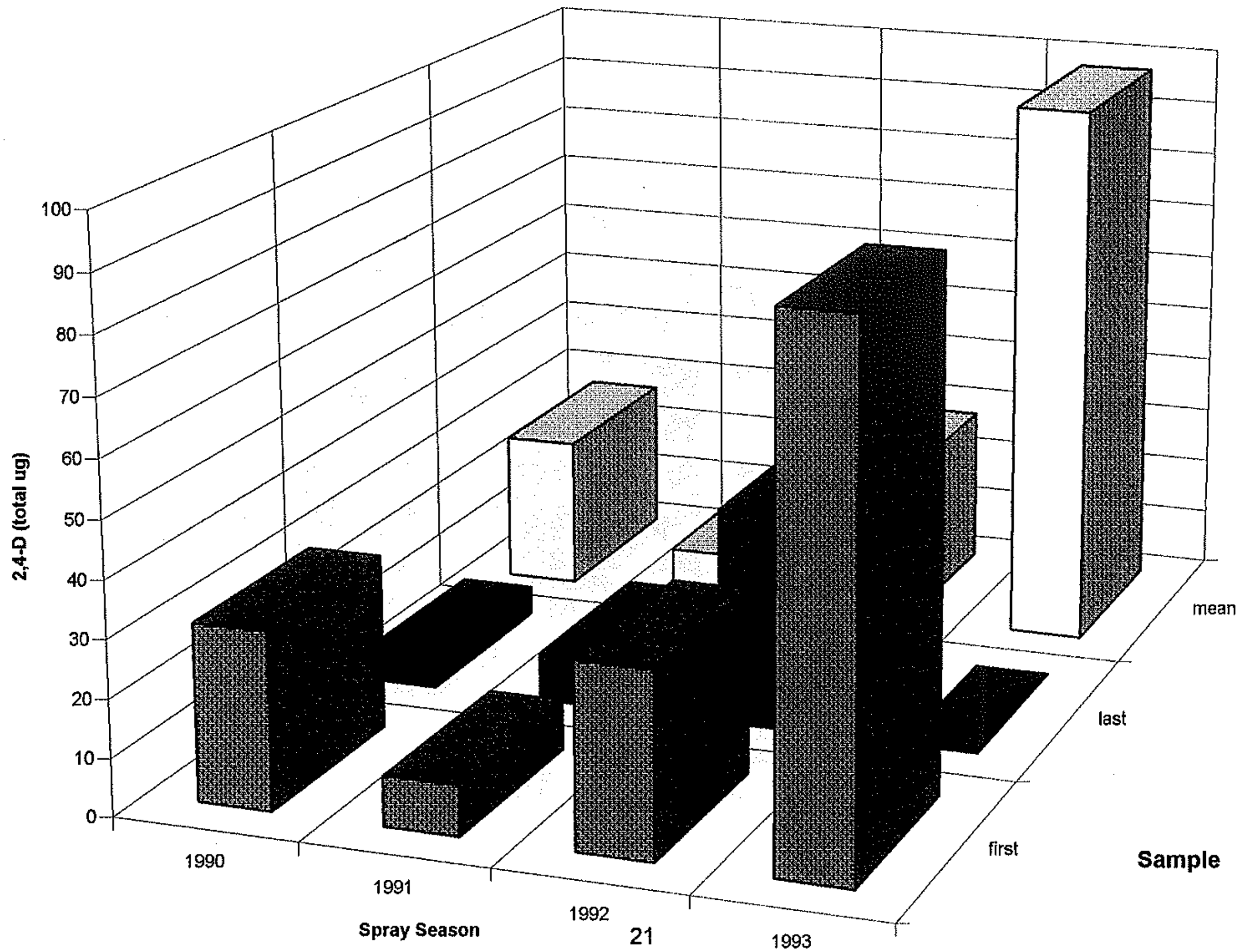


Fig 9. Trend of 2,4-D Levels in Urine of Worker 9 During Four Spray Seasons



instituted and wash up facilities had been provided. Work often depended on weather, with some weeks having less rain and therefore more spraying activity. The varied amount of activity can explain the significance of the Week variable.

Since the variables Site and Day were expected to be significant, these variables were further investigated through linear regression analysis. The independent variables, Site and Day, were controlled for in test of the interactive terms, Site and Day. No significant effect was evident (p=2.00). Because the interaction effect was not significant, any Site effect is consistent across all days and the Day effect is consistent at all sites. Additionally, Site was not significant when Day was controlled for (p=0.87). Day was not significant when Site was controlled for (p=1.14).

Site and Day were expected to be significant on the basis of known exposure and excretion rates of 2,4-D and Triclopyr. A higher concentration of 2,4-D was applied so workers had a greater risk of exposure to 2,4-D than Triclopyr. Secondly, 2,4-D has been found to be more efficiently adsorbed dermally than Triclopyr (Feldman and Maibach, 1974; Carmichael et al., 1989). The Sunday sample, taken 2 to 3 days after last exposure was expected to show far less total chlorinated herbicide than the work day samples.

In order to compare values noted in this study to safe levels, an arithmetic mean of 2,4-D in urine was determined for the seasonal samples. Since spraying was performed June through October, the mean was then adjusted to reflect an annual level i.e, 5 work days per week, 4.5 weeks per month, 3.5 months per year or 79 work days per year. This amounted to 22 percent of a year (79 days/365 days). All means were multiplied by 0.22 and this value, in total micrograms (ug), was compared to the Reference Dose which required conversion of the standard units of mg/kg/day to micrograms. The RfD is 0.01 mg/kg/day. This value was converted to 700 ug by multiplying by an average body weight of 70 kg. All annual levels of 2,4-D in urine were far below the RfD of 700 ug.

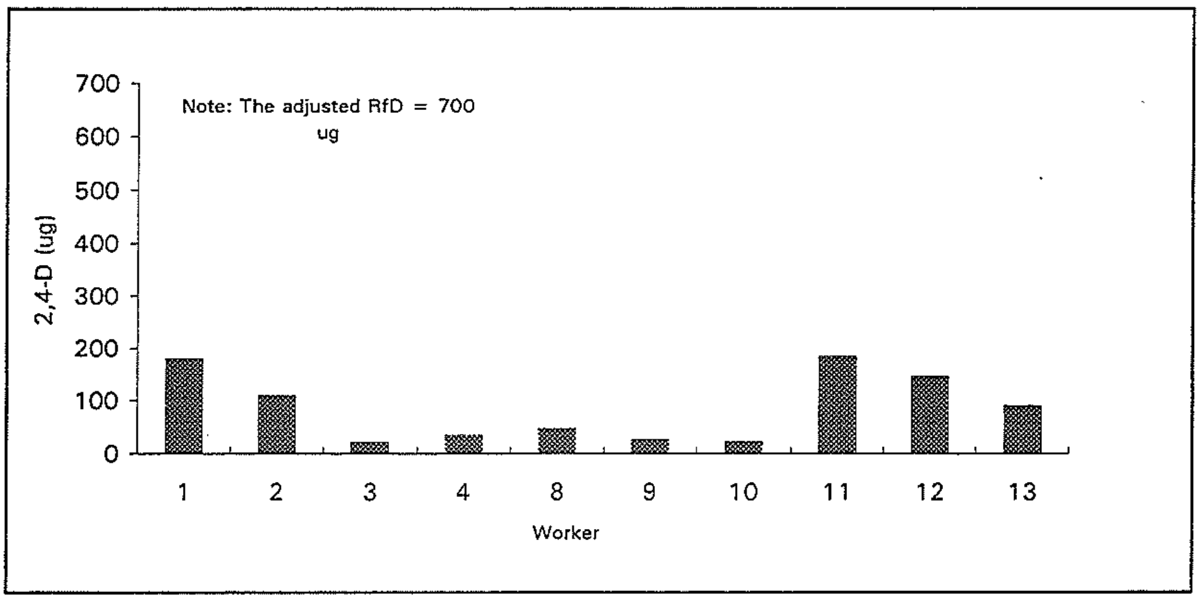


Figure 12. Comparison of adjusted mean 2,4-D amount in worker urine to adjusted RfD.

Moreover, results obtained in this study clearly show that 2,4-D did not bioaccumulate in the tissues of field applicators over time as shown in figures 1, 2, 3, and 4. A similar conclusion can be made for workers applying Triclopyr in Hammond (Figures 5, 6 and 7) indicating the low potential of accumulation of both 2,4-D and Triclopyr in tissues and organs of workers spraying these herbicides.

In comparison to previous literature values for 2,4-D in worker urine, the Tulane study values were in the same approximate range. Lavy et al. (1982) observed a maximum of 4200 ug 2,4-D in forestry ground workers while Knopp and Glass (1991) found a maximum of 5950 ug in agricultural workers. Tulane maximums ranged from 819 to 1824 ug in Baton Rouge and 212 to 2306 ug in Bridge City workers. These values are presented in Table 2.

Table 2. Maximum 2,4-D Levels Observed in Daily Urine Samples

Occupation	Total ug excreted	Reference
forestry, ground	4200	Lavy et al., 1982
forestry, helicopter	1156	Lavy et al., 1982
agriculture	5950	Knopp and Glass, 1991
agriculture	280	Nash et al., 1982
airboat	645	Nigg and Stamper, 1987
roadside, Baton Rouge	1824 max. 22 - 181 mean	Tulane study
roadside, Bridge City	2306 max. 23 - 184 mean	Tulane study

b. Triclopyr in Urine

Maximum Triclopyr levels in urine for the Hammond group ranged from not detected to 438 ug Triclopyr. These maximum values were lower than the 2,4-D maximum values. The differences can be explained by the fact that Triclopyr has stronger herbicidal properties than 2,4-D, thus the Triclopyr solution used (0.84 acid equivalent/hectare) was approximately one half the concentration of 2,4-D (2.1 acid equivalents/hectare) used in the field. Also, whereas dermally applied 2,4-D is absorbed at 5.2% (Feldman and Maibach, 1974), only 1.7% of dermally applied Triclopyr is absorbed (Carmichael, 1989). Consequently, even if equal amounts of 2,4-D and Triclopyr were deposited on the skin, more 2,4-D would penetrate and be eventually found in the urine.

No study of occupational exposure to Triclopyr was found in the literature. No RfD or Drinking Water Maximum Contaminant Level exists, so values must be compared to agricultural tolerances. Agricultural tolerances for picloram, a close relative of Triclopyr, allow up to 0.05 ppm in eggs, 0.5 ppm in grains and 0.2-5 ppm in meats for human consumption. Triclopyr at below 500 ppm is accepted in animal forage. A 0.01 ppm concentration is allowed in milk. Concentrations of 0.05 to 0.5 ppm are accepted in animal meat.

For lack of other values for comparison, the Hammond results were compared to a lethal dose(LD_{50}) which kills 50% of test animals

(LD_{50}). The lowest LD_{50} in the literature is a rat value of 630 mg/kg body weight (HSDB, 1994). The maximum Triclopyr value of 43 ug, when converted to a dose equals 6.1E-4 mg/kg body weight (0.043mg/70kg body weight). This value is vastly lower than the rat LD_{50}. The level of Triclopyr in urine has a margin of safety of more than 1000 times the LD_{50}.

c. Glyphosate in Urine

The total amount of glyphosate excreted in urine on working and non-working days ranged from nondetectable to a maximum of 175ug. As with Triclopyr, there is little information available from occupational exposure studies. A single study of forestry workers exposure to glyphosate found glyphosate in urine at 0.085 mg/l. Five workers were monitored during a single week of work (Jauhiainen et al., 1991). The detection limit in this experiment was 100 ppb while the Tulane detection limit was 0.1 pcb. At a detection limit of 100 pcb, all of the Tulane samples would have been reported as nondetectable. In the Jauhiainen method, the sample was reduced to five times the original concentration while the Tulane method concentrated a sample either 12 or 25 times the original concentration.

The data were plotted on histograms which showed that the natural logarithm values were normally distributed. All statistical analysis utilized the ln transformed data. Bivariate analysis was conducted using ANOVA to determine whether the variables, Worker, Week, Site, Herbicide and Day of the Week, were independently predictive of the amount of herbicide in urine.

Table 3. Results of ANOVA of Glyphosate in Urine

VARIABLE	F	p value
Person	$F_{12,151}$	0.2905
Week	$F_{17,146}$	0.48
Site	$F_{2,161}$	0.814
Day	$F_{4,152}$	0.26

None of the variables person, week, Site, or day were predictors of amount of glyphosate in urine (Table 3). Since the variables Site and Day were expected to be significant, they were further investigated through linear regression analysis. The independent variables, site and day, were controlled in the test of the interactive terms, site and day. No significant effect was evident (p=0.343). Additionally, Day was not significant when Site was controlled for (p=0.561). Similarly, Site was not significant when Day was controlled for (p=1.47).

The chemical structure of glyphosate indicates that dermal adsorption is unlikely. The kinetics of dermal uptake of glyphosate has not been published. Furthermore, rat studies have shown that some metabolism of ingested or injected glyphosate can occur and that excretion routes include both urine and feces (USEPA, 1986b). Given the lack of information on glyphosate kinetics, no specific exposure patterns were predicted at the start of this experiment. As was done with 2,4-D, the total ug of glyphosate in urine was adjusted to reflect that work was performed only 22% of the year. These values were then compared to the proposed RfD (2.0 mg/kg/day) (Federal Register, 8/18/93) which was converted to a microgram value of 140,000 ug. All amounts of glyphosate were at least 10,000 times less than the RfD (Figure 3).

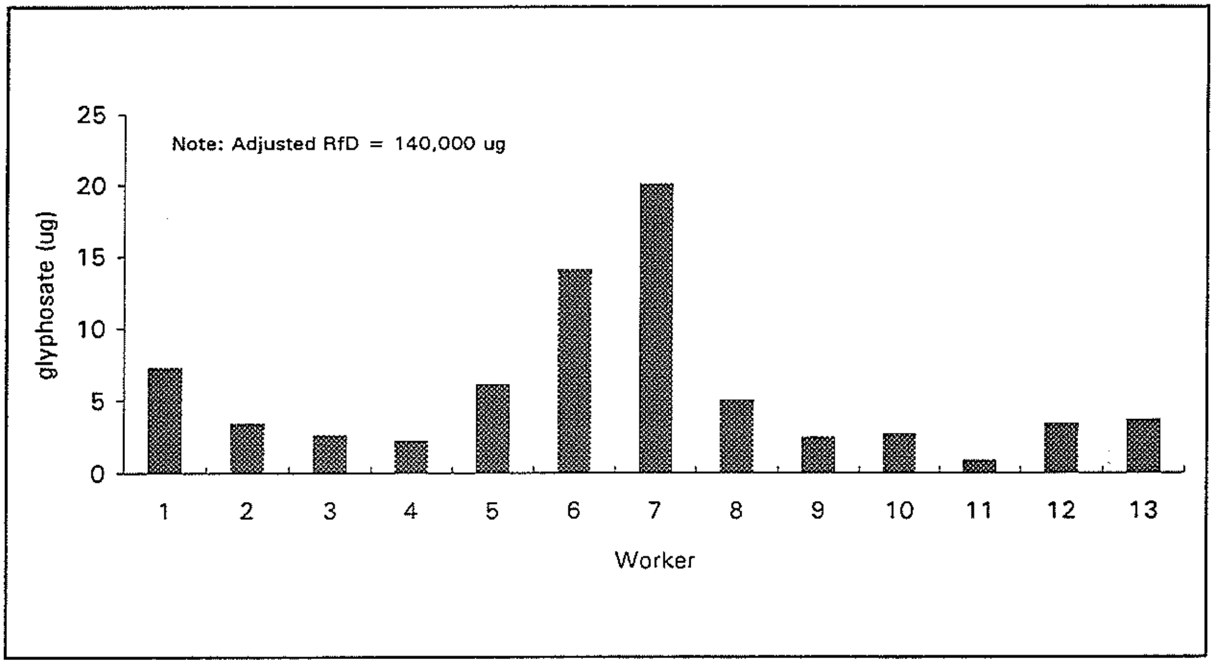


Figure 13. Comparison of adjusted mean glyphosate amount in worker urine to adjusted RfD.

d. Mixtures

Under working conditions found at various field locations, the herbicide applicators receive an acceptably low level of exposure to the three herbicides: 2,4-D, Triclopyr and glyphosate. No adverse health effects due to exposure to the individual herbicides is expected. Furthermore, the possibility of toxic effects from the mixture is also highly unlikely given the current knowledge about the use of these herbicides.

Both 2,4-D and Triclopyr are rapidly excreted as the parent compound from the body (greater than 95% within 24 hours) (Gehring et al., 1973; Feldman and Maibach, 1974; Carmichael et al., 1989). The short residence time of the chlorinated herbicides curtails the chance of interaction with glyphosate. Often, it is the metabolite rather than the parent compound which generates a toxic response. With the chlorinated herbicides, there is no metabolite available to interact with glyphosate.

Although the kinetics of glyphosate is less well known, it is also rapidly excreted in animals, (90% excreted within 48 hours by rats). Since glyphosate has been found in tissues of farm animals, there is a chance that absorption and even metabolism may occur in humans. On the basis of the small amounts of herbicides which enter the body, the rapid rate of excretion of the herbicides and the minor presence of metabolites, there is little opportunity for a potential adverse interaction between 2,4-D or Triclopyr and glyphosate to occur.

3. Air Sampling Results

Table 4 presents a summary of both chlorinated herbicides and glyphosate data, including number of air samples analyzed and the maximum and minimum values obtained.

4. Discussion

a. Concentrations of 2,4-D in Air

Inhalation amounts of 2,4-D ranged from nondetectable (less than 0.003 ug) to 64.0 ug inhaled during a work period. These values were compared to the findings of other researchers to determine if the LADOTD workers were receiving typical amounts through inhalation. Literature values were presented in a variety of units, with concentration in air volume (ug/cum) being the most common.

To make comparisons, literature values were converted to total micrograms inhaled during the day (Table 5). If hours of work were not specified in the literature, an 8 hour working day was assumed. The Tulane values were within the range reported by other investigators and well below the OSHA Threshold Weighted Average(TWA)(10 mg/cubic meter). The maximum Tulane value reported was 100 times less than the converted TWA as shown in Table 5.

Table 4. Summary of Herbicides Collected in Air Samples (June 19, 1990 to October 9, 1990)

ID	Chlorinated herbicide			Glyphosate		
	Minimum	Maximum	No. samples	Minimum	Maximum	No. samples
	(ug)	(ug)		(ug)	(ug)	
BR 1	<0.003	16.5	20	<0.1	65.2	17
BR 2	<0.003	9.8	19	<0.1	67.9	19
BR 3	<0.003	3.3	12	<0.1	2.8	5
BR 4	0.6	48.5	11	<0.1	11.3	8
HD 5	0.1	4.5	5 (Tri)	<0.1	138.6	5
HD 6	0.3	1.5	5 (Tri)	<0.1	19.4	3
HD 7	0.2	34.6	5 (Tri)	<0.1	35.8	5
BC 8	1.3	16.5	6	<0.1	7.3	6
BC 9	1.6	13.7	8	1.1	6.4	7
BC 10	<0.003	41.5	4	0.5	2.7	4
BC 11	<0.003	64	16	<0.1	8.3	17
BC 12	<0.003	49.2	16	<0.1	80.8	12
BC 13	<0.003	0.9	4	<0.1	11.6	2

Note: BR- Baton Rouge
BC - Bridge City
HD - Hammond
Tri - Triclopyr

Table 5. Comparison of 2,4-D Inhalation Exposures

Reference	Occupation	Air concentration (mg/cu m)	Total mg inhaled
OSHA TWA	not specified	10	64
Manninen, et al., 1986	farmer tractor	1E-3 to 2E-1	6E-3 to 1.3
Libich et al., 1984	backpack spray	4E-4 to 9.1E-2	3E-3 to 5.8E-1
Lavy et al., 1982	forest	0.06 ug/l	3.5E-2
Lavy et al., 1982	forest, helicopter	nd-0.13 ug total	1.3E-4
Kolmodin Hedman, 1983	farmer	1E-6 to 1E-4	6E-6 to 6E-4
Nigg and Stamper, 1987	airboat appli-cation	1E-3 to 3E-3 inhaled/hour	8E-3 to 2.4E-2
DOT, 1994	roadside	NA	3E-6 to 6.4E-2

Note: No study was found which reported levels of Triclopyr in air during application.
Conversion : 10 mg/cu m x 0.8 cu/hr x 8 hour/day

b. Concentrations of Triclopyr in Air

No study of levels of Triclopyr in the breathing zone of applicators was found in the literature. Since the Triclopyr solution was more diluted than 2,4-D, it was expected that workers would not inhale as much. Workers 5 and 6 from Hammond inhaled maximum Triclopyr levels of 5 and 2 ug, respectively. Worker 7, however, had a higher maximum level of 35 ug which exceeded the 2,4-D maximum values for 3 out of 4 Baton Rouge workers (Workers 1, 2 and 3; maximums of 3.3 to 17 ug), and for 3 out of 6 Bridge City workers (workers 8, 9 and 13; maximums of 0.9-17 ug).

c. Concentrations of Glyphosate in Air

A single study of worker exposure to glyphosate was found. In this week-long study of 5 workers, glyphosate was found in two air samples at 2.8 and 15.7 ug/cu.m. The detection limit was 1.25 ug/cum (Juahiainen et al., 1991). When Tulane study results are expressed as concentration in air rather than amount inhaled by worker, the maximum air concentration found (Worker 5, 33.45(ng) on 7/23/90) was 17.9 ug/cu.m. This value was very similar to that reported by Juahiainen et al. (1991).

d. Mixtures

Workers were not exposed to excessive air concentrations of any of the herbicides. Additionally, levels of the three herbicides were well below values associated with toxicity found in the literature. On the basis of the small amounts of herbicide which enter the body, the rapid rate of excretion of the herbicides and the minor presence of metabolites, there is little opportunity for a potential adverse interaction between 2,4-D or Triclopyr and glyphosate to occur.

B. TOXICITY TESTING

1. Fish/Crawfish Bioassays

Toxicity data indicated that the surfactant appeared to be the most toxic chemical against catfish and bluegills, while 2,4-D was the least toxic. Garlon 3A was more toxic than 2,4-D and less toxic than Round-up. On effect, data showed 96-hrs lethal concentration(LC_{50}) which kills 50% of test animals values of 3.6 $\pm$ 0.2 mg/L, 14.5 $\pm$ 1.2 mg/L, 344.3 $\pm$ 20.6 mg/L and 451.9 $\pm$ 82.6 mg/L for the surfactant, Round-up, Garlon-3A and 2,4-D, respectively. Thus, the order of decreasing toxicity was: Surfactant > Roundup > Garlon-3A > 2,4-D. However, an inverse situation was observed in bioassays with crawfish where Garlon-3A was found to be less toxic than 2,4-D and more toxic than Round-up. In this experiment, 96-hrs LC_{50} values of 64,002.4 $\pm$ 14,923.7 mg/L, 20,117.9 $\pm$ 1,073.0 mg/L and 1,870.3 $\pm$ 16.0 mg/L were recorded for Round-up, Garlon-3A and 2,4-D, respectively.

Among the three species of aquatic organisms, catfish and bluegill sunfish seemed to have similar levels of sensitivity to individual chemicals. No significant difference ($p>0.05$) was observed between the mean values of 96-hrs LC_{50} of any tested chemical on catfish compared to bluegills. However, significant differences ($p<0.05$) were obtained when comparing catfish and bluegills data to those of crawfish indicating that crawfish were less sensitive to chemical toxicity than catfish and bluegills.

Studies carried out to assess the toxicities of Roundup, Garlon-3A and 2,4-D in dual mixtures using channel catfish and bluegill sunfish as test organisms also indicated combined toxic effects either simply additive or slightly antagonistic.

2. Microbial toxicity assessment

Results of bioassays using microorganisms indicated that among the three tested herbicides, Roundup was the most inhibitory compound on microbial growth and respiration, while 2,4-D was the least inhibitory one. Toxicity results of various herbicide mixtures to microorganisms indicated that mixtures of Roundup and 2,4-D or of Roundup and Garlon-3A provided combined toxic effects which were simply additive and/or slightly antagonistic.

C. SOIL ADSORPTION/DESORPTION STUDIES

Soils analyses were performed by the LTRC laboratory at the LADOT research central facility in Baton Rouge. Testing included analysis with #4, 10, 40 and 200 sieves, percent clay and silt analysis (by hydrometer), and Atterburg limits.

Baton Rouge soil had the highest iron and clay content as well as a pH (pH < 8) which provided a positively charged iron molecule. Therefore, the Baton Rouge soil should adsorb more glyphosate than the other soils. This capacity was evidenced in the isotherm study by the higher K_f value for the Baton Rouge soil $_f$(K =8.7). In comparison, Bridge City and Hammond had K_f values of 0.1 and 0.34, respectively. The Hammond soil had both less iron (pH < 8) and less clay than Baton Rouge but still adsorbed greater than 90% of the glyphosate. At the concentrations used in this study, the capacity of the clay in the Hammond soil was not exceeded.

A higher 1/n value indicates a greater capacity for amount adsorbed per weight of adsorbate as the equilibrium concentration increases. In this study, Bridge City soil had a slope of 1/n equal to 0.92 while Baton Rouge soil had a 1/n value of 0.43 and Hammond had a 1/n value of 0.75. The Bridge City and Hammond soils appeared to have a similar capacity. They were very close as a result of both capacity and pH.

In the desorption isotherm study, values for K_d were 355, 0.04 and 0.005 ug/g for Baton Rouge, Bridge City and Hammond, respectively. A far greater amount of glyphosate desorbed from the Baton Rouge soil than from the Hammond or Bridge City soil. The addition of water for the desorption study altered the equilibrium between bound and unbound glyphosate, and glyphosate was released. Since the Baton Rouge soil had the highest adsorption K_f value, there was more glyphosate available for release than from the Hammond or Bridge City soils.

D. ENVIRONMENTAL SAMPLES

The levels of glyphosate found in the vegetation samples were compared to Food and Drug Administration (FDA) Action Levels for grain (0.1 ppm) as this sample matrix was the most similar to vegetation. Glyphosate in vegetation exceeded the Action Level of 0.1 ppm during the summer sampling dates (July through October).

No water sample exceeded the Drinking Water Maximum Contaminant Level (MCL) of 0.700 ppm for glyphosate. Limits and advisories for plyphosate concentrations in soil and sediment do not exist.

Levels of 2,4-D in Baton Rouge and Bridge City 0 inches soil samples ranged from less than detection limits (<0.02 ppm) to 0.43 ppm, and less than detection limits (<0.02 ppm) to 0.39 ppm, respectively. In Baton Rouge and Bridge City, trace levels of 2,4-D (0.01 ppm) remained in the soil through the winter of 1990/1991 but decreased to levels below detection prior to the start of the 1992 spray season. In the Baton Rouge 3 inches soil samples, 2,4-D was detected in 6 of 10 analyzed samples (controls not included). In the Bridge City samples, it was found in 3 of 15 analyses. The maximum concentrations detected were 0.09 ppm and 0.05 ppm in Baton Rouge and Bridge City, respectively.

In the analysis of sediment field samples, no 2,4-D was detected in the Baton Rouge samples. A trace amount of 2,4-D was detected in June and October, 1991 two Bridge City samples at less than 0.003 ppm.

Field water samples from Baton Rouge had detectable levels of 2,4-D at less than 0.002 ppm in 2 of 16 samples. The maximum concentration of 2,4-D in water samples was 0.002 ppm. The maximum concentration in Bridge City waters was 0.026 ppm. In Bridge City water samples, 2,4 -D was also infrequently detected in 5 out of 12 samples.

In conclusion, 2,4-D, as used by the LADOTD, has shown no potential to accumulate in the roadside environment. Levels of

2,4-D in vegetation were compared to the FDA Action Level for grain. The vegetation exceeded this Action Level throughout the spray season and into April, 1991 in Baton Rouge vegetation and April, 1992 in Bridge City vegetation. Concentrations of 2,4-D were compared to the Maximum Contaminant Level(MCL) for 2,4-D in drinking water of 0.1 mg/L. Canal water in Bridge City and Baton Rouge had 2,4-D concentrations consistently below 0.01 mg/L.

In soils, the physical behavior of the herbicides applied as a mixture is unchanged from that when one herbicide is used. The previous discussion of sorption studies identified that the three herbicides in question follow the same sorption patterns when in a mixture as they do when applied independently. Results from the field sampling show that glyphosate and 2,4-D concentrations in surface soil decrease to below detectable levels prior to each spray season. Glyphosate and 2,4-D were still detected in vegetation samples because these included a mix of leaf litter (with stored herbicide) and new growth. A small amount of Triclopyr was detected in soil six months after spraying at 0.03 ppm. Triclopyr is the most persistent of the three herbicides.

The duration of the herbicides in the Louisiana roadside environment correspond to values described in the literature. Unpublished studies by Tulane University have shown that the toxicity of the mixtures to soil organisms is no greater than when the herbicides are used alone. Therefore, since the mixtures do not increase the persistence of the herbicides in the roadside environment nor cause an increased toxic effects to soil microorganisms, the use of 2,4-D / glyphosate and Triclopyr/glyphosate mixtures appears to few or little adverse environmental effects.

E. MICROCOSM STUDY

1. Microcosm Description

The Baton Rouge microcosm had a vegetative cover of thick 4 inches (10cm) tall grass. A mat of dead grass littered the soil surface which was 12(30cm) by 24(61cm) inches in area. The soil slope in the microcosm was approximately 25-30 degrees. A visibly different clay layer comprised the subsurface soil. The water which was 3(8cm) to 4(10cm) inches deep, was clear and following agitation, rapidly returned to clear conditions. The sediment layer was roughly 1(2.5cm) inch deep.

The Bridge City microcosm had a 12 by 24 inches (30 by 60 cm) soil sloped at approximately 20 degrees. The vegetative cover was thick, short and comprised of 1 to 3 inches (2.5-7.5 cm) grasses and broadleaved weed species. The water was 3 to 4 (7.5-10 cm) inches deep and clear but colored. The sediment layer was roughly 1 inch (2.5cm)in depth.

The Hammond microcosm soil surface was 12 by 24 (30 by 60 cm) inches and had approximately a 40 degree slope. The vegetative cover consisted of thick 1 to 2 inches (2.5-5 cm) diameter clumps of grass approximately 6-12 (15-30 cm) in height interspersed with several 24 inches (61 cm) tall woody weed species. The water was 3 to 4 inches (7.5-10 cm) deep and was continuously dark in color.

in microcosm vegetation at 0 hour (185 ppm) was well below the field vegetation sample at 0 hour (500 ppm). By Week 1 the levels in microcosm and field samples merged. In Baton Rouge, the microcosm mean was 11 ppm while the field sample was 37 ppm. In Bridge City a microcosm value of 8 ppm approached the field result of 43 ppm.

At Week 1, 2,4-D was not detected in any of the extracted Baton Rouge or Bridge City subsurface samples. When compared to field samples, 2,4-D was never found in sediment or water samples.

The physical behavior of the herbicides applied as a mixture is unchanged from that when one herbicide is used. The previous discussion of sorption studies identified that the three herbicides in question follow the same sorption patterns when in a mixture as they do when applied independently. It is the soil composition, organic content, clay, pH and iron content rather than the herbicide which regulates sorption. Any alteration to soil sorption which could occur would require vastly more concentrated mixtures than those used by the LADOTD. A spill, for example, could introduce a volume of herbicide which could cause a localized change in soil pH. Also the surfactant, which is added to the mixture to promote movement of the herbicide through the plant tissue could, in high concentrations, influence water solubility of the herbicides and thereby influence soil sorption characteristics. In conclusion, no significant effect upon the physical or chemical integrations between soil and herbicide is expected to result from the normal use of a mixture of herbicides.

CONCLUSIONS

1- Results of the study indicated that exposure of workers to mixtures of herbicides via inhalation was insignificant compared to threshold limit values set by the American Conference of Governmental Industrial Hygienists (ACGIH), the National Institute for Occupational Safety and Health (NIOSH) and the Occupational Safety and Health Administration (OSHA).

2- Herbicide levels in the urine of these workers were much higher than those found in breathing air. This was probably due to exposure via ingestion (eating without proper hand washing) or to exposure via dermal contact.

3- The trend of levels of herbicides in the urine of workers during four spray seasons indicated a slight increase during each season. However,urine results showed no herbicide carry over from one spray season to another.

4- The analysis of environmental samples including sediment and water collected over three years indicated that 2,4-D, Roundup and Garlon-3A did not pose an immediate (acute) risk to aquatic life in water bodies adjacent to herbicide treated areas on the right-of-way.

5- Acute toxicity testing of fish, crawfish and microorganisms showed that toxic levels were not reached with herbicide application at the manufacturers recommended rates.

6- Soil studies indicated that Roundup has an higher capacity to adsorb to Louisiana soils than the 2,4-D and Triclopyr.

RECOMMENDATIONS

a. For human and ecosystem health protection

While low levels of herbicides were detected in samples collected from applicators and the, care should be taken in their application:

1) In this study, low concentrations of 2,4-D and Garlon 3A (Triclopyr) were detected in the air collected from breathing zones. Although the levels were much below the ACGIH recommended threshold limit values (TLVs), precautions should be taken. All herbicide applicators should stay inside the cab and windows should be kept closed during spraying.

2) Herbicide concentrations were much higher in urine than in air. This means that herbicides entered the body from routes other than inhalation, possibly through ingestion or absorption through the skin. Hands must be properly washed after handling the herbicides especially before meals. Protective clothing should be worn and spills on skin must be avoided.

3) To avoid aerosol inhalation,applicators should stand upwind of the prevailing wind direction when mixing the herbicides. Spraying rigs should be modified (if and when possible) so nozzles are located at the back of the truck.

4) Results of herbicide mixtures testing on fish and crawfish indicate low acute toxicity values, however, this does not preclude toxicity to other aquatic species. Chronic toxicity to all aquatic life forms may also be of concern. Therefore, spills and direct application of herbicides to water should be avoided.

b. There is a need for future research:

1) To assess The potential exposure to herbicide of the Mobil Equipment Operators of open mowing tractors. The operators could be exposed via inhalation and/or dermal contact by contaminated grass dust particles.

2) To determine the extent and impact of herbicides' drift.

3) To evaluate subchronic and chronic effects of these herbicides on fresh water organisms.

4) To examine the bioavailability of these compounds to life forms living in treated areas.

5) To study the fate of grass particles resulting from mowing immediately. Rain and wind may carry contaminated cut grass to adjacent water bodies causing possible water and soil contamination.

REFERENCES

1. Alsop, G.M., Waggy, G.T. and Convay, R.A., Bacterial growth inhibition test. Journal of Water Pollution Control Federation, 52 (10): 2452-2456, 1980.

2. American Public Health Association. Standard Methods for the Examination of Water and Wastewater, 18th Edition. APHA, AWWA, WEF. Washington D.C., 1992.

3. Anderson, A.C., Abdelghani, A.A., Tchounwou, P.B. and Monkiedje, A., Interpretation of microbial bioassays using kinetic parameters. Journal of Environmental Science and Health, Part B: Pesticides, Food Contaminants and Agricultural Wastes, **B23**(3): 211-223, 1988.

4. Audus, L.J.(ed). Herbicides, Physiology, Biochemistry and Ecology. Academic Press, London, 1976.

5. Carmichael, N.G., Perkins, J.M., Davies, R., and Warrington, S.J., Oral and dermal pharmacokinetics of triclopyr in human volunteers. Human toxicology, **8**: 431-437, 1989.

6. Carter, M (ed). Soil Sampling and Methods of Analysis. Canadian Society of Soil Analysis. Lewis Publishers, 1993.

7. Feldman, R.J. and Maibach, H., Percutaneous penetration of some pesticides and herbicides in man. Toxicology and Applied Pharmacology, **28**: 126-132, 1974.

8. Gehring, P.J., Kramer, C.G., Schwetz, B.A., Rose, J.Q. and Rowe, V.K., Toxicol. Appl. Pharmacol, **26**: 352, 1973.

9. Harris, S.A and Salomon, K.R., Human exposure to 2,4-D following controlled activities on recently sprayed turf. Journal of Environmental Science and Health, **27**(1): 9-22,1992.

10. National Library of Medicine. Hazardous Substance Data Bank, 1994.

11. Jauhiainen, A., Rasanen, K. Sarantila, R. Nuutinen, J. Kangas,J., Occupational Exposure of forest workers to glyphosate during brush saw spraying work. American Industrial Hygiene Association Journal, **52**: 61-64, 1991.

12. Knoop, D., and Glass S., Biological monitoring of 2,4-D acid exposed workers in agriculture and forestry. Institute for Water, Soil and Air Hygiene. Federal Health Office Research Department, Federal Republic of Germany, 1991.

13. Kolmodin-Hedman, B., Hoglund, S. and Akerblom, M., Studies on phenoxyacid herbicides. Archives of Toxicology, **54**: 257-265, 1983.

14. Lavy, T.L., Norris, L.A., Mattice, J.D. and Marx, D.B., Exposure of Forestry Ground Workers to 2,4-D, Picloram and Dichlorop. Environmental Toxicology and Chemistry, **6**: 209-224, 1987.

15. Lavy, T.L., Shepard, J.S. and Mattice J.D., Exposure measurements of applicators spraying (2,4,5- Trichlorophenoxy acetic acid) in the forest. Journal of Agriculture and Food Chemistry, **28**: 626-630, 1987.

16. Lavy, T.L. and Mattice, J.D., Monitoring human exposure during pesticide application in the forest. Chapter 21 Chemical and Bilogical Controls in Forestry, American Chemical Society Symposium Series p 238, 1989.

17. Lavy, T., Walstad, J., Flynn, R. and Mattice, J., 2,4 Dichlorophenoxyacetic acid exposure received by aerial application crews during forest spray operations. Journal of Agricultural Food Chemistry, **30**: 375-381, 1982.

18. Libich, S., To, J., Frank, R. and Sirons, G., 1. Occupational exposure of herbicide applicators to herbicides used along electric power line right-of-ways. American Industrial Hygiene Association, **45**: 56-62, 1984.

19. Marking, L.L., Toxicity of chemical mixtures. "In Fundamentals of Aquatic Toxicology". Rand and Petrocelli (eds). Hemisphere Publishing Corporation, Washington, 1985.

20. Metcalf, M.C., The Fate of herbicide mixtures (2,4-D, Triclopyr and Glyphosate) in humans and the environment. Doctoral thesis. Tulane University, New Orleans La, 1994.

21. Murphy, S.D., Toxic Effects of Pesticides, Klassen, C.D., Amdur, M.O. and Doull J., (eds). Macmillan Publishing Company, New York, 1986.

22. National Institute of Occupational Safety and Health, Method 5001, 2,4-D and 2,4,5-T, issued 1984.

23. Ribo, J.M. and Kaiser, L.E., Photobacterium phosphoreum toxicity bioassay. I. Test procedures and applications. II. Toxicity data compilation. Toxicity Assessment-An International Journal, **2**: 195-237, 1987.

24. Sittig, M., Agricultural Chemicals Manufacture, Noyes Data Corporation, Park Ridge, New Jersey, 1971.

25. Torstensson, N.T., Lundgren, L.N. and Stenstrom, J., Influence of climatic and edaphic factors on persistence of glyphosate and 2,4-D in forest soils. Ecotoxicology and Environmental Safety, **18**: 230-239, 1989.

26. U.S. Environmental Protection Agency, Determination of glyphosate in wastewater, Method 127, Effluent Guidelines Division, 1983.

27. U.S. Environmental Protection Agency, Methods for measuring the acute toxicity of effeluents to fresh water and marine organisms, 3rd ed. EPA /600/4-85 /013, Environmental Monitoring and Support Laboratory, Cincinnati, Oh, 1985.

28. U. S. Environmental Protection Agency, Office of Pesticide Programs, Registration Division, Fact Sheet -Glyphosate, Report No. 540/FS-88-124, 1986.

29. U.S. Environmental Protection Agency, June, Office of Pesticide Programs, Guidance for the re-registration of pesticide products containing glyphosate as the active ingredient, Case Number 0178, Report No. 540/RS-86-154, 1986.

30. U.S. Environmental Protection Agency, Test Methods for Evaluating Solid Wastes, USEPA Office of Solid Waste and Emergency Response. Washington D.C., 1994.

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