EXCRETION BALANCE, METABOLIC FATE, AND
TISSUE RESIDUES OF N-PHENYL-
N'-1,2,5-THIADIAZOL-3-YLUREA (PHOTOTHIDIAZURON)
IN RATS

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Terry L. Johnson
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TABLE OF CONTENTS

CHAPTER	PAGE
I. INTRODUCTION	1
II. REVIEW OF LITERATURE	3
Structure, Nomenclature, and Physicochemical Properties	
of Photothidiazuron and Thidiazuron	3
Photoconversion of Thidiazuron to Photothidiazuron . . .	5
Toxicity, Activity and Uses, and Mode of Action of	
Thidiazuron	5
Mammalian Metabolism of Substituted Urea Pesticides . . .	6
III. MATERIALS AND METHODS	11
Compounds	11
Chromatography and Autoradiography	13
Radioisotopic Methodology	15
<u>In Vivo</u> Studies of Photothidiazuron	15
Treating and Handling of Rats	15
Analysis of Urine	16
Analysis of Feces	17
Analysis of Tissues	17
<u>In Vitro</u> Studies of Photothidiazuron	18
Degradation by Rat Liver Subcellular Fractions	18
Degradation by a Model Hydroxylating System	19
IV. RESULTS	21
<u>In Vivo</u> Studies of Photothidiazuron	21

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CHAPTER	PAGE
IV. RESULTS (continued)	
Excretion Balance Following Treatment of Rats with Photothidiazuron- ¹⁴ C	21
Metabolic Fate Following Treatment of Rats with Photothidiazuron- ¹⁴ C	21
Tissue Residues Following Treatment of Rats with Photothidiazuron- ¹⁴ C	29
<u>In Vitro</u> Studies of Photothidiazuron	29
Degradation of Photothidiazuron- ¹⁴ C by Rat Liver Subcellular Fractions	29
Degradation of Photothidiazuron- ¹⁴ C by a Model Hydroxylating System	34
V. DISCUSSION	37
VI. SUMMARY	41
LITERATURE CITED	43

LIST OF TABLES

TABLE	PAGE
I. Chromatographic Behavior of Photothidiazuron and Related Compounds	14
II. Cumulative Percentage of Photothidiazuron- ¹⁴ C-Equivalents Eliminated in the Urine and Feces of Male Rats	22
III. Elimination of Radiocarbon in the Urine and Feces of Male Rats Treated Orally with Photothidiazuron- ¹⁴ C Expressed as Percentage of Administered Radioactivity	23
IV. Results of Fractionation of Urine from Male Rats Treated Orally with Photothidiazuron- ¹⁴ C	24
V. Nature and Relative Concentration of Organosoluble Radio- active Material Isolated from the Urine of Male Rats Treated Orally with Photothidiazuron- ¹⁴ C	26
VI. Hydrolysis of Water-Soluble Radioactive Material Isolated From Urine Samples of Male Rats Treated Orally with Photothidiazuron- ¹⁴ C when Incubated for 18 Hours with Enzymes and Acid	28
VII. Nature and Concentration of the Released Aglycone Moiety Resulting from Enzymatic and Acidic Cleavage of Radio- active Water-Soluble Compounds Isolated from Urine of Male Rats Treated Orally with Photothidiazuron- ¹⁴ C	30
VIII. Results of Fractionation of Feces from Male Rats Treated Orally with Photothidiazuron- ¹⁴ C	31

TABLE	PAGE
IX. Concentration of Radioactive Material in the Tissues of Male Rats 96 Hours After Oral Treatment with Photothidiazuron- ¹⁴ C	32
X. Distribution of Radiocarbon-Containing Compounds Following Degradation of Photothidiazuron- ¹⁴ C by Rat Liver Subcellular Fractions	33
XI. Nature and Relative Concentration of Organosoluble Radio- carbon-Containing Compounds Following Degradation of Photothidiazuron- ¹⁴ C by Rat Liver Subcellular Fractions	35
XII. Nature and Relative Concentration of Radiocarbon-Containing Compounds Following Degradation of Photothidiazuron- ¹⁴ C by a Model Hydroxylating System	36

LIST OF FIGURES

FIGURE	PAGE
1. Conversion of Thidiazuron to Photothidiazuron	4
2. Autoradiograph Showing Radiochemical Purity of Photo- thidiazuron- ¹⁴ C	12
3. Diagrammatic Representation of Two-Dimensional Chromatogram Obtained Following TLC of Organosoluble Radioactive Material Isolated from Urine of Male Rats Treated Orally with Photothidiazuron- ¹⁴ C	25
4. Paths for Photothidiazuron Metabolism by Rats	39

CHAPTER I

INTRODUCTION

Thidiazuron (DROPP[®]) or N-phenyl-N'-1,2,3-thiadiazol-5-ylurea is a promising new cotton defoliant currently being developed by NOR-AM Agricultural Products, Incorporated. In addition to its defoliating activity, thidiazuron demonstrates strong inhibition of regrowth with no adverse effect on the opening or quality of fiber of mature bolls not open at the time of application (Anonymous, 1975). These attributes indicate this could be a useful product.

With the possibility of widespread use of thidiazuron in cotton growing regions, it is necessary to consider the result of its photodegradation. The major thidiazuron conversion product, which occurs on the plant surface, is photothidiazuron or N-phenyl-N'-1,2,5-thiadiazol-3-ylurea (Anonymous, 1976). This new compound is the result of a rearrangement in the thiadiazol moiety (Anonymous, 1976).

Thidiazuron and photothidiazuron are classified chemically as substituted ureas. This includes a broad group of compounds varying greatly in their molecular substituents and commercial uses. However, most substituted ureas are herbicides, and therefore, the orientation of research has been directed to plant and soil situations. Research data pertaining to the effects of substituted ureas upon mammalian and other animal systems is very limited (Geissbühler, et al., 1975). Since it is possible that leaves and other parts of a thidiazuron treated cotton plant may be incorporated into livestock feed or eaten by other animals, it is

essential to examine certain of the properties of photothidiazuron in relation to mammalian systems.

The purpose of this study was to determine the excretion balance, metabolic fate, and tissue residues of photothidiazuron in rats.

Specifically, the objectives were:

1. To determine the excretion balance, metabolic fate, and tissue residues after the administration of a single oral dose of photothidiazuron-aniline-¹⁴C to male rats,
2. To investigate the degradation of photothidiazuron by rat liver subcellular fractions and by a model hydroxylation system, and
3. To identify major photothidiazuron metabolites.

CHAPTER II

REVIEW OF LITERATURE

STRUCTURE, NOMENCLATURE, AND PHYSICOCHEMICAL PROPERTIES OF PHOTOTHIDIAZURON AND THIDIAZURON

Photothidiazuron is N-phenyl-N'-1,2,5-thiadiazol-3-ylurea (Figure 1). It is the major photoconversion product of thidiazuron, a cotton defoliant being developed in the United States by NOR-AM Agricultural Products, Incorporated, Woodstock, Illinois (Anonymous, 1976).

Photothidiazuron is a yellowish, crystalline powder of molecular weight 220.3. This compound is soluble in water at approximately 41 to 46 mg/L. It is stable in both acidic and alkaline solutions (pH 1 to pH 14). Treatment by either acid (1 N HCl at 90°C for 4 hours) or base (1 N NaOH at 90°C for 4 hours) yielded no hydrolytic products (Anonymous, 1977).

Thidiazuron is a white, odorless, crystalline material. It has a molecular weight of 220.3 and a melting point of 217°C with decomposition (Anonymous, 1975). Solubility characteristics of thidiazuron indicate that it is slightly soluble in water (20 ppm), chloroform (13 ppm), benzene (35 ppm) and hexane (6 ppm). In other solvents the solubility in g per liter of solvent is >500 in dimethyl sulfoxide and dimethylformamide, 215 in cyclohexane, 4.6 in methanol, 8 in acetone and 0.8 in ethyl acetate (Anonymous, 1975). Under extreme acid conditions (pH 1), thidiazuron showed no decomposition after 4 hours (Anonymous, 1976). Under basic conditions (1 N sodium hydroxide and 90°C) there was some

Figure 1. Conversion of thidiazuron to photothidiazuron.

hydrolysis of the molecule with the formation of aniline (Anonymous, 1977).

PHOTOCONVERSION OF THIDIAZURON TO PHOTOTHIDIAZURON

In the presence of light the thiadiazole moiety rearranges from the 1,2,3-thiadiazole-5-yl form of thidiazuron to the 1,2,5-thiadiazole-3-yl form of photothidiazuron (Figure 1). This reaction takes place mainly on the leaf surface to which thidiazuron has been applied (Anonymous, 1977).

TOXICITY, ACTIVITY AND USES, AND MODE OF ACTION OF THIDIAZURON

Thidiazuron has demonstrated low toxicity to mammals, birds, and fish. In its proposed formulation, 50% wettable powder, thidiazuron yielded acute oral LD₅₀ values of >4000 mg/kg in rats, 3900 mg/kg in male mice, 2600 mg/kg in female mice, and >9980 mg/kg in Japanese quail. The acute dermal LD₅₀ value for mammals is >1000 mg/kg in both rabbits and rats. Acute inhalation LC₅₀ (4 hours) value of technical thidiazuron for rats is 72.3 mg/L. Thidiazuron caused slight skin and eye irritation in rabbits after 28 days exposure (Anonymous, 1975).

Thidiazuron's defoliant activity is due to the induction of abscission resulting in the dropping of non-dessicated green leaves. It defoliates at a rate of 50 to 250 g active ingredient per hectare (Arndt et al., 1976). Thidiazuron rivals organophosphate defoliants at recommended rates and gives less rapid regrowth. Cotton bolls are not affected by this compound and open normally if mature at the time of application. Activity is temperature related; temperatures below 70°F increase time for defoliation (Anonymous, 1975).

Thidiazuron exhibits growth regulating activity such as decreased fruit maturation time. It is one of several 1,2,3-thiadiazole derivatives invented for this purpose (Schulz and Arndt, 1975). Other effects of thidiazuron on plants include stem thickening, shortening of the internodes, increased branching, and general growth retardation and stunting with application of large amounts (Anonymous, 1975).

Future uses of thidiazuron other than cotton defoliation may utilize its growth regulatory activity. Possible situations for use could be in the delaying of flowering for frost protection, increased sugar content of sugarcane and beets, and defoliation of fruit and other crops for mechanical harvest (Anonymous, 1975).

MAMMALIAN METABOLISM OF SUBSTITUTED UREA PESTICIDES

Mammalian metabolism of substituted urea herbicides and general considerations of substituted urea herbicides was reviewed by Geissbühler et al. (1975). Rats dosed orally with phenylurea herbicides labeled with radiocarbon in various positions, such as chloroxuron-carbonyl- ^{14}C , chlorbromuron-carbonyl- ^{14}C , metobromuron-ring- ^{14}C , and chlortoluron-tolyl- ^{14}C , showed rapid and efficient excretion of the radioactive material in urine and feces. Greater than 90% of the administered dose was excreted within 72 hours. Most of the radiocarbon appeared in urine with less than 20% of the administered dose appearing in the feces. Elimination rate was not appreciably affected by either dose or sex (Geissbühler et al., 1975). About 93% of the radiocarbon from thidiazuron-aniline- ^{14}C and thidiazuron-thiadiazole- ^{14}C administered orally to rats was excreted in the urine and feces in 95 hours. Up to 56% of the radiocarbon was excreted in the feces (Crecelius and Knowles, 1978). In goats treated with thidiazuron- ^{14}C for

10 days, the majority of radiocarbon was excreted in the urine and feces (>70%) with more than 50% of the dose appearing in the urine (Benezet et al., 1978).

Phenylureas and their metabolites do not accumulate in body tissues and fluids. Analysis of blood and other tissues showed that <1% of the administered dose remained after 72 hours (Geissbühler et al., 1975). Tissues from rats treated with a single oral dose of thidiazuron-¹⁴C had <20 ppb of radiocarbon (Crecelius and Knowles, 1978). Tissue residues in rats on a dietary supplement of 6.6 ppm of thidiazuron-¹⁴C for 9 days showed a rapid increase at the onset of the experiment followed by a plateau which remained until rats were replaced on an untreated diet. Stomach and intestine tissues reached radiocarbon levels of >200 ppb, and kidney, blood, and liver contained 100 to 200 ppb. Heart, brain, spleen, uterus, fat, and muscle contained <100 ppb. By the seventh day on untreated food more than 50% of the radiocarbon in the tissues had cleared (Crecelius and Knowles, 1978). Radiocarbon-containing residues in tissues from goats and hens treated with multiple doses of thidiazuron-¹⁴C also were low (Benezet et al., 1978).

Two major biochemical transformation pathways in mammals have been established for phenylurea herbicides. N-Demethylation of monuron and diuron was indicated as the main pathway of urea herbicide metabolism. Analysis of rat urine showed greater quantities of unsubstituted urea metabolites than of monomethylated metabolites. This suggests that dealkylation was a primary reaction in mammalian tissues. N-Dealkoxylation was a major reaction with linuron and monolinuron (Bohme and Ernst, 1965; Ernst and Bohme, 1965; Geissbühler et al., 1965).

Phenylurea herbicides show great similarity in regard to their mammalian metabolism. It has been demonstrated that most urinary metabolites retain the urea moiety, and many are in the form of hydroxylated derivatives. All the herbicides tested were altered to some degree by the animal (Ernst and Bohme, 1965). It was noted that compounds with monochlorine substituents at the para position yielded primarily meta-hydroxylated derivatives; dichloro compounds gave 6-hydroxy derivatives, and all were subject to conjugation as glucuronides and ethereal sulfates (Geissbühler et al., 1975). Studies with other compounds structurally related to the phenylureas, such as 3,4,4'-trichlorocarbamilide and 3-trifluoromethyl-4,4'-dichlorocarbamilide, demonstrated similar metabolic reactions (Jeffcoat et al., 1977; Birch et al., 1978; Hiles and Birch, 1978; Warren et al., 1978).

Ring hydroxylation was demonstrated in fluometuron, metobromuron, linuron, monolinuron and chlorbromuron (Geissbühler et al., 1975). Major metabolites of linuron and monolinuron were hydroxylated compounds (Ernst and Bohme, 1965; Bohme and Ernst, 1965; Geissbühler et al., 1975). Crecelius and Knowles (1978) and Benezet et al. (1978) reported ring hydroxylation of thidiazuron in rats, goats and chickens.

In a study of siduron metabolism in dogs and rats it was found that ring hydroxylation was the primary biotransformation pathway. The administered compound formed 1-(p-hydroxyphenyl)-3-(2-methylcyclohexyl)-urea, 1-(4-hydroxy-2-methylcyclohexyl)-3-(p-hydroxyphenyl)urea and 1-(4-hydroxy-2-methylcyclohexyl)-3-phenylurea. As an index of the hydroxylating ability of rats and dogs, their respective urine was analyzed for total content of siduron metabolites. Dog urine contained more aniline-yielding residues than rat urine and less para-aminophenol-yielding

residues indicating that the rat system hydroxylated more efficiently than the dog (Belasco and Reiser, 1969).

Biotransformation of chlortoluron in rats consisted mainly of oxidation of the methyl ring moiety to the corresponding hydroxymethyl intermediate and then to the carboxy derivative. Chlortoluron is the only phenylurea herbicide thus far to have demonstrated this pathway (Geissbühler et al., 1975).

Studies of phenylurea herbicides have shown that the formation of aniline by hydrolysis of parent herbicide or metabolites was not a major mammalian pathway. A two year feeding study of diuron with rats and dogs showed only traces of aniline metabolites in the urine (Hodge et al., 1967).

When chloroxuron-carbonyl- ^{14}C and chlorbromuron-carbonyl- ^{14}C were administered to rats 0.1 to 2% of the radioactivity was recovered as carbon dioxide in expired air (Geissbühler et al., 1975). The same range was found for thidiazuron- ^{14}C in rats in vivo and in vitro (Benezet and Knowles, 1978).

Enzyme hydrolysis experiments revealed that phenylurea metabolites were conjugated as glucuronides and ethereal sulfates in mammals. With the exception of ring-hydroxylated compounds, retention of the intact aniline moiety by all conjugates of the phenylureas and their metabolites was a trend (Hodge et al., 1967; Geissbühler et al., 1975). Rats treated with thidiazuron- ^{14}C also conjugated certain of the radiocarbon-containing metabolites as glucuronides and sulfates (Crecelius and Knowles, 1978).

Diflubenzuron is a substituted urea with insecticidal activity. In insects it inhibits the synthesis of chitin. Published research

indicated that the behavior of diflubenzuron in mammals was much like that of its related substituted urea herbicides. When lactating cattle were treated orally with diflubenzuron- ^{14}C 85% of the administered radioactivity was excreted in the feces and 15% in the urine. Milk samples taken at 24 hours showed a maximum radiocarbon content of 0.8 ppm. Tissue residues after seven days were low with only the liver showing appreciable amounts (2.3 to 3.6 ppm) (Ivie, 1978).

Metabolites of diflubenzuron were similar to those derived from urea herbicides. Ring hydroxylation was the major metabolic pathway along with cleavage between the carbonyl and amide moieties. Most metabolites were conjugated as glucuronides or sulfates (Ivie, 1978).

In summation, substituted urea herbicides and insecticides are easily absorbed, metabolized and rapidly excreted in the urine and feces of mammals. Tissue and body fluid residues of substituted ureas were low and short lived following either single dose or dietary supplement administration. Biotransformation of substituted urea compounds containing a N-methyl group or a N-methoxy group was by N-demethylation and N-dealkoxylation, respectively. Compounds containing ring moieties underwent ring hydroxylation followed by conjugation as glucuronides and sulfates as the major pathways of biotransformation.

CHAPTER III

MATERIALS AND METHODS

COMPOUNDS

A radiolabeled sample of photothidiazuron was provided by NOR-AM Agricultural Products, Incorporated, Woodstock, Illinois. Photothidiazuron-aniline- ^{14}C , designated hereinafter as photothidiazuron- ^{14}C , was uniformly labeled in the aniline ring with a specific activity of 10.59 mCi/mmole. The sample was determined by thin-layer chromatography and autoradiography to be greater than 99% radiochemically pure (Figure 2).

Also provided by NOR-AM were nonradioactive samples of photothidiazuron, N-4-hydroxyphenyl-N'-1,2,5-thiadiazol-3-ylurea or 4-hydroxyphenylphotothidiazuron, thidiazuron, and N-2-hydroxyphenyl-N'-1-2-3-thiadiazol-5-ylurea or 2-hydroxyphenylthidiazuron. The latter compound was dissolved in methanol and treated with sunlight to yield 2-hydroxyphenylphotothidiazuron. Samples of phenylurea, 2-hydroxyacetanilide, 3-hydroxyacetanilide, and 4-hydroxyacetanilide were purchased from Aldrich Chemical Company, Incorporated, Milwaukee, Wisconsin. Aniline, 2-aminophenol, 3-aminophenol, 4-aminophenol, and acetanilide were obtained from Fisher Scientific Company, St. Louis, Missouri. 4-Hydroxyphenylurea was synthesized from 4-aminophenolhydrochloride as described by Kalckhoff (1883) (melting point: 167-8°C found; 168°C reported).

Figure 2. Autoradiograph showing radiochemical purity of photothidiazuron- ^{14}C .

CHROMATOGRAPHY AND AUTORADIOGRAPHY

Resolution, purification and tentative identification of photothidiazuron- ^{14}C and its radiolabeled metabolites was accomplished by thin-layer chromatography (TLC). Glass TLC plates (20 x 20 x 0.25 cm) were coated with a 500- μ layer of silica gel GF₂₅₄ (Brinkmann Instruments, Incorporated, Des Plaines, Illinois). The silica gel coated plates were activated at 100°C for one hour and kept in a dessicator at room temperature until used.

Aliquots from organosoluble fractions were spotted on TLC plates with capillary tubes (0.7 to 1.0 mm inside diameter), and the chromatograms were developed to a height of at least 15 cm for adequate separation. Also spotted on the TLC plates were nonradioactive standards. Each chromatogram was developed two-dimensionally. The first solvent system was ethyl acetate; the second system was a chloroform-ethyl acetate (1:1) mixture. The TLC development tanks were equilibrated for at least one hour before use, aided by the placement of absorbent paper along one side of the tank. Chromatographic characteristics of photothidiazuron and related compounds are given in Table I.

Upon completion of TLC, each plate was placed in contact with no-screen X-ray film (Eastman Kodak Company, Rochester, New York) and exposed for 15 to 30 days depending upon the amount of activity present on the chromatogram. The autoradiographs were used to locate radiocarbon labeled compounds. The nonradioactive standards were located on the TLC plate by observation under short wavelength ultraviolet light (254 nm).

Quantitation of photothidiazuron and its metabolites was achieved by scraping the silica gel from TLC plates at spots corresponding to the

TABLE I
CHROMATOGRAPHIC BEHAVIOR OF PHOTOTHIDIAZURON
AND RELATED COMPOUNDS^a

Compound	Average R _f value in solvent system	
	A	B
Photothidiazuron	0.74	0.67
2-Hydroxyphenylphotothidiazuron	0.68	0.53
4-Hydroxyphenylphotothidiazuron	0.64	0.40
Thidiazuron	0.45	0.20
Acetanilide	0.52	0.37
2-Hydroxyacetanilide	0.56	0.39
3-Hydroxyacetanilide	0.48	0.20
4-Hydroxyacetanilide	0.43	0.16
Aniline	0.68	0.60
2-Aminophenol	0.62	0.38
3-Aminophenol	0.60	0.31
4-Aminophenol	0.48	0.26
Phenylurea	0.39	0.17
4-Hydroxyphenylurea	0.23	0.04

^aTLC was accomplished on glass plates coated with a 500-micron layer of silica gel GF₂₅₄. The following solvent systems were used:

A = ethyl acetate and B = chloroform-ethyl acetate (1:1).

exposure spots on the X-ray film into scintillation vials. After the addition of 10 mL of scintillation cocktail to each vial the mixtures were radioassayed.

RADIOISOTOPIC METHODOLOGY

The radiocarbon content of each sample was measured with a Liquimat 200 liquid scintillation spectrometer (Picker Nuclear, White Plains, New York). The counting efficiency for radiocarbon was 98.0%. Most radioactive samples were placed in glass scintillation vials with 10 mL of scintillation cocktail and counted. The amount of radioactivity was calculated by averaging duplicate sample counts. All data were corrected for background, dilution, quenching, and counting efficiency. Scintillation cocktail for these assays consisted of 1.5 L each of toluene and methylcellosolve plus 2,5-diphenyloxazole (15g) and 1,4-bis-2-(4-methyl-5-phenyloxazole) (0.9g).

Radioactive samples obtained from the combustion of feces and tissue samples were assayed with Permafluor[®] V scintillation cocktail (Packard Instruments Company, Incorporated, Downers Grove, Illinois).

IN VIVO STUDIES OF PHOTOTHIDIAZURON

Treating and Handling of Rats

Male albino rats (Sprague-Dawley Strain) weighing about 150 g each were obtained from Charles River Company, Boston, Massachusetts. Each rat was treated via stomach tube with approximately 3 μ Ci of photothidiazuron-¹⁴C dissolved in acetone and Carbowax[®] PEG 400 (Fisher Scientific Company) (1:2). The dosage level corresponded to about 0.24 mg/kg. Following treatment each rat was placed in a modified Roth metabolism

chamber for 96 hours. Food and water were provided ad libitum. Urine and fecal samples were collected separately at intervals of 3, 12, 18, 24, 72, and 96 hours after treatment.

Analysis of Urine

Total radioactivity in each sample of whole urine was determined by radioassay of duplicate 50 μ L aliquots. The remaining urine was extracted three times with equal volumes of ethyl acetate. The ethyl acetate extracts were combined and dried over anhydrous sodium sulfate. It was then reduced to a volume of 1 mL under air, and duplicate 50 μ L aliquots were radioassayed to determine the concentration of radioactivity in the organic fraction. The remaining extract was concentrated to about 0.1 mL and subjected to TLC, autoradiography and radioassay for identification and quantitation of the radioactive components. Duplicate 50 μ L aliquots of each urine sample remaining after ethyl acetate extraction were radioassayed to determine radioactivity in the water fraction (Crecelius and Knowles, 1978).

To study the nature of radioactive material remaining in the water fraction, some samples were treated with beta-glucuronidase, aryl sulfatase and HCl following the procedure of Sen Gupta and Knowles (1970). A 0.2 mL to 0.5 mL aliquot (depending on sample activity) of extracted urine was placed in a 25 mL Erlenmeyer flask with 4 mL of citrate-phosphate buffer (0.1 M citric acid monohydrate, 0.2 M Na_2HPO_4 , pH 5.0, Kuhr and Casida, 1967), and 3 mg of either beta-glucuronidase (15% salt form, Type B-1: bovine liver, 500,000 Fishman Units per g, Sigma Chemical Company, St. Louis, Missouri) or aryl sulfatase (salt-free form, Type V: from limpets, 5-10 units per mg, Sigma Chemical Company). The HCl treatment

flask contained the urine aliquot, buffer and HCl to yield a final concentration of 9% (v/v). The control consisted of the designated amount of extracted urine and 4 mL of citrate-phosphate buffer. The samples were incubated for 18 hours at 38°C in a Dubnoff metabolic shaking incubator (Precision Scientific Company, Chicago, Illinois). Following incubation the reaction was stopped by the addition of 1 mL of ethyl acetate. The HCl mixture was neutralized with concentrated sodium carbonate. The flask contents were extracted three times with equal volumes of ethyl acetate. The extracts were combined, dried over anhydrous sodium sulfate, and evaporated to a volume of 1 mL. Duplicate 50 µL aliquots were taken for radioassay. The remaining extracts were reduced to 0.1 mL and subjected to TLC, autoradiography and radioassay for identification and quantitation (Crecelius and Knowles, 1978).

Analysis of Feces

Feces from each sampling interval were dried under an Infra-Radiator[®] light (Fisher Scientific Company) and ground to a powder with mortar and pestle. Total radioactivity in each sample was determined by combustion of duplicate 100 mg portions using a Model 306 Tri-Carb Sample Oxidizer (Packard Instrument Company, Incorporated). The $^{14}\text{CO}_2$ resulting from the combustion was trapped in 4 mL of Carbo-Sorb[®] (Packard Instrument Company, Incorporated) and added to 13 mL of Permafluor V scintillation fluid for radioassay. Ground feces samples were placed directly into Combusto Cones^R (Packard Instrument Company, Incorporated) for combustion.

Analysis of Tissues

Rats were sacrificed after 96 hours, and blood samples were taken from the heart with a 1 mL syringe. Selected tissues were removed and

weighed wet. Duplicate 100 mg samples of each tissue were wrapped in small pieces of Kimwipes[®] (Hi-Count[®], Type 900-S, Kimberly-Clark Corporation, Neenah, Wisconsin) and placed in Combusto Cones (Crecelius and Knowles, 1978). Fat tissue samples were ground by mortar and pestle with an equal weight of cellulose powder prior to wrapping. Blood samples were applied directly to Combusto Pads[®] (Packard Instrument Company, Incorporated) in Combusto Cones. Total radioactivity in the tissues was subsequently determined by combustion and radioassay as described for fecal samples.

IN VITRO STUDIES OF PHOTOTHIDIAZURON

Degradation by Rat Liver Subcellular Fractions

Liver was dissected from 150 g to 180 g male rats (Sprague-Dawley). A homogenate was prepared by slicing the liver into small pieces with a razor blade and washing with 0.25 M sucrose (2-4°C). The pieces of liver were homogenized in 25 mL of 0.25 M sucrose solution (2-4°C) in a glass homogenizer with a teflon pestle. The homogenate was centrifuged at low speed to remove extraneous material and cellular debris. Aliquots of the supernatant were taken as samples of crude homogenate. The remaining homogenate was fractioned into subcellular components by differential centrifugation (Ahmad and Knowles, 1970). The following fractions were prepared: 600 g for 15 minutes precipitate (nuclear fraction), 11,000 g for 20 minutes precipitate (mitochondrial fraction), 96,000 g for 60 minutes precipitate (microsomal fraction), and 96,000 g for 60 minutes supernatant (soluble fraction). Precipitated pellets were washed and resuspended to their original volume with 0.25 M sucrose (2-4°C).

A standard incubation mixture consisted of 1 mL of 0.1 M tris-HCl buffer (pH 7.4), 1 mL of a respective rat liver fraction and 200,000 dpm of photothidiazuron- ^{14}C dissolved in 10 μL of acetone. Some of the mixtures also contained 2 μmoles of NADPH (Sigma Chemical Company). Control mixtures contained all components except the subcellular fraction and were analyzed simultaneously. The reaction mixtures were incubated aerobically in 10 mL Erlenmeyer flasks at 38°C for 1 hour with shaking (Ahmad and Knowles, 1971). The reaction was terminated by the addition of 1 mL of ethyl acetate. The mixtures were extracted three times with equal volumes of ethyl acetate. The organic extracts were combined, dried over anhydrous sodium sulfate, and concentrated with air to 1 mL. Duplicate 50 μL aliquots of the water and ethyl acetate fractions were taken for radioassay (Crecelius and Knowles, 1978). The ethyl acetate fraction was analyzed further by TLC, autoradiography, and radioassay.

Degradation by a Model Hydroxylating System

Into 25 mL Erlenmeyer flasks were placed 200,000 dpm of photothidiazuron- ^{14}C , 80 μmoles of EDTA, 142 μmoles of ascorbic acid, 15 μmoles of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ and 3 mL of 0.1 M phosphate buffer (pH 5.5) (Udenfriend *et al.*, 1954). These flasks and appropriate controls were incubated in air at 38°C for 5 hours with constant shaking. After 3 hours of incubation an additional 142 μmoles of ascorbic acid in 0.5 mL of buffer were added. Incubation was terminated by the addition of 1 mL of ethyl acetate to each flask (Crecelius and Knowles, 1978).

Flask contents were extracted three times with equal volumes of ethyl acetate, the extracts were combined and dried over anhydrous sodium sulfate. After concentration under air to a volume of 1 mL, duplicate

50 μ L aliquots of the organic extract were taken for radioassay. The remainder was reduced to 0.1 mL and subjected to TLC, autoradiography, and radioassay. Duplicate 50 μ L aliquots of the water fraction of each flask also were radioassayed (Crecelius and Knowles, 1978).

CHAPTER IV

RESULTS

IN VIVO STUDIES OF PHOTOTHIDIAZURON

Excretion Balance Following Treatment of Rats with Photothidiazuron- ^{14}C

When a single oral dose of photothidiazuron- ^{14}C was administered to rats 75.5% of the dose was excreted in the urine by 96 hours after treatment (Table II). An additional 15.2% was eliminated in the feces during this interval (Table II). Thus by 96 hours approximately 90.7% of the administered dose was eliminated in the urine and feces (Table II). The highest level of photothidiazuron- ^{14}C equivalents was recorded at 12 hours posttreatment in urine (36.3%) and at 24 hours posttreatment in the feces (5.6%) (Table III).

Metabolic Fate Following Treatment of Rats with Photothidiazuron- ^{14}C

Extracting the urine with ethyl acetate removed from 4.6% (3-12 hours) to 10.2% (72-96 hours) of the radioactive material (Table IV). Separation of this organosoluble urinary radioactive material by TLC yielded at least 15 compounds plus material at the origin (Figure 3, Table V). The material at the origin accounted for 36.3% of the radioactivity at 3 hours posttreatment and decreased to 2.9% after 96 hours. Compounds I, II, and III also showed a general decrease with time. Compound IV increased from 3.1% at 3 hours to a peak of 10.2% at 18 hours and then decreased to 3.3% at 96 hours posttreatment. Compound V, which cochromatographed with 4-hydroxyphenylurea, ranged from 1.1% at 3 hours

TABLE II
 CUMULATIVE PERCENTAGE OF PHOTOTHIDIAZURON-¹⁴C EQUIVALENTS
 ELIMINATED IN THE URINE AND FECES OF MALE RATS^a

Time Interval Following Treatment (H)	Cumulative % of Dose		
	Urine	Feces	Total
0-3	20.3 (12.4)	0.1 (0.05)	20.4
3-12	56.6 (8.3)	0.3 (0.1)	56.9
12-18	61.5 (6.7)	3.5 (2.3)	65.0
18-24	67.9 (6.0)	9.1 (3.9)	77.0
24-48	73.1 (4.6)	13.8 (3.4)	86.9
48-72	74.8 (5.0)	14.9 (3.4)	89.7
72-96	75.5 (5.1)	15.2 (3.6)	90.7

^aData are means from at least 3 rats per time interval. Standard deviation is given in parentheses.

TABLE III

ELIMINATION OF RADIOCARBON IN THE URINE AND FECES OF MALE RATS
TREATED ORALLY WITH PHOTOTHIDIAZURON- ^{14}C EXPRESSED AS
PERCENTAGE OF ADMINISTERED RADIOACTIVITY^a

Time Interval Following Treatment (H)	Urine	Feces	Total
0-3	20.3 (12.4)	0.1 (0.05)	20.4
3-12	36.3 (11.1)	0.2 (0.15)	36.5
12-18	4.9 (3.9)	3.2 (2.1)	8.1
18-24	6.4 (5.2)	5.6 (3.2)	12.0
24-48	5.2 (3.7)	4.7 (2.7)	9.9
48-72	1.7 (0.9)	1.1 (0.8)	2.8
72-96	0.7 (0.3)	0.3 (0.2)	1.0
Total	75.5	15.2	90.7

^aData are means from at least 3 rats per time interval. Standard deviation is given in parentheses.

TABLE IV
RESULTS OF FRACTIONATION OF URINE FROM MALE RATS
TREATED ORALLY WITH PHOTOTHIDIAZURON- ^{14}C ^a

Time Interval Following Treatment (H)	% Radioactivity	
	Organic Phase	Aqueous Phase
0-3	5.1 (0.9)	94.9 (0.9)
3-12	4.6 (0.5)	95.4 (0.5)
12-24	6.0 (2.2)	94.0 (2.2)
24-48	10.0 (5.2)	90.0 (5.2)
48-72	9.6 (4.9)	90.4 (4.9)
72-96	10.2 (4.5)	89.8 (4.5)

^aData are means from at least 3 rats per time interval.
Standard deviation is given in parentheses.

Figure 3. Diagrammatic representation of two-dimensional chromatogram obtained following TLC of organosoluble radioactive material isolated from urine of male rats treated orally with photothidiazuron- ^{14}C . Roman numerals and hatched zones designate radioactive metabolites. Arabic numerals and clear zones designate authentic standards as follows: 1=phenylurea, 2=4-hydroxyacetanilide, 3=thidiazuron, 4=3-hydroxyacetanilide, 5=4-aminophenol, 6=acetanilide, 7=2-hydroxyacetanilide, 8=3-aminophenol, 9=2-aminophenol, 10=4-hydroxyphenylphotothidiazuron, 11=aniline, 12=photothidiazuron, 13=4-hydroxyphenylurea, and 14=2-hydroxyphenylphotothidiazuron.

TABLE V

NATURE AND RELATIVE CONCENTRATION OF ORGANOSOLUBLE RADIOACTIVE MATERIAL ISOLATED FROM THE URINE OF MALE RATS TREATED ORALLY WITH PHOTOTHIDIAZURON- $^{14}\text{C}^a$

Compound	% Radioactive Material at Indicated Time Interval (H)						
	0-3	3-12	12-18	18-24	24-48	48-72	72-96
Origin	36.3	17.6	7.1	11.1	5.3	2.6	2.9
I	17.6	7.7	5.0	4.0	2.7	1.9	1.9
II	5.2	2.9	<0.1	<0.1	<0.1	<0.1	<0.1
III	6.3	7.6	7.4	2.8	3.0	3.1	4.2
IV	3.1	6.7	10.2	6.0	5.2	4.6	3.3
V	1.1	1.8	3.3	18.2	12.7	10.6	7.0
VI	0.9	<0.1	0.6	4.3	1.8	<0.1	<0.1
VII	6.7	6.3	8.6	6.2	11.1	12.8	9.8
VIII	1.5	4.8	2.2	1.6	2.7	2.6	3.4
IX	3.3	12.3	6.7	5.0	4.7	7.7	6.4
X	3.5	15.1	13.0	16.8	8.8	10.3	8.5
XI	0.9	1.3	<0.1	<0.1	1.4	0.5	2.0
XII	4.5	9.4	6.8	9.3	7.0	13.0	13.8
XIII	6.5	2.0	4.9	3.9	6.4	13.5	9.8
XIV	0.9	1.2	0.7	2.8	0.4	1.0	0.9
XV	1.7	3.3	23.5	8.0	26.8	15.8	26.1

^aCompounds were separated by two-dimensional TLC on glass plates coated with a 500- μ layer of silica gel GF₂₅₄; the solvent systems were ethyl acetate (first direction) and chloroform-ethyl acetate (1:1) (second direction). Compounds V, VII, VIII, XIII, and XV cochromatographed with 4-hydroxyphenylurea, phenylurea, 4-hydroxyacetanilide, 4-hydroxyphenylphototidiazuron, and phototidiazuron, respectively. Compounds V, VIII, XII and XIII also yielded a positive reaction in chromagenic tests for aromatic hydroxyl moiety (Krishna *et al.*, 1962).

to a maximum of 18.2% at 24 hours and decreased to 7.0% of the organo-soluble radioactivity after 96 hours. Compound VII, which cochromatographed with phenylurea, comprised 6.7% of the organosoluble radioactive material at 3 hours, reached a maximum level of 12.8% at 72 hours, and decreased to 9.8% by 96 hours. Compound VIII, which cochromatographed with 4-hydroxyacetanilide, attained its highest level (4.8%) after 12 hours as did Compound IX (12.3%). Compound X comprised 15.1% after 12 hours, 13.0% after 18 hours and 16.8% after 24 hours. Compound XII showed a general increase from 4.5% after 3 hours to 13.8% by 96 hours posttreatment. Compound XIII, which cochromatographed with 4-hydroxy-phenylphotothidiazuron, ranged from 2.0% at 12 hours posttreatment to 13.5% after 96 hours. Photothidiazuron- ^{14}C , compound XV, was present at relatively high levels (>15%) at all time intervals with the exception of 3 and 24 hours (Table V).

The vast majority (>90%, Table IV) of the radioactive material remained in the urine after ethyl acetate extraction, and the results of hydrolysis of this material by enzymes and acid are given in Table VI. The largest amount of radioactive materials released into the organic phase resulted from HCl treatment. During the 0-12 hour interval the HCl treatment yielded 30.5% in the organic phase and 69.5% in the aqueous phase. Beta-Glucuronidase yielded maximum cleavage of 15.2% in the organic phase at the 12-24 hour interval. Aryl sulfatase showed the least amount of cleavage yielding a high of only 9.3% radioactivity in the organic phase at the 12-24 hour time interval (Table VI).

The nature and concentration of the released aglycone moiety resulting from enzymatic and acidic cleavage of radioactive water-soluble compounds

TABLE VI

HYDROLYSIS OF WATER-SOLUBLE RADIOACTIVE MATERIAL ISOLATED FROM URINE
 SAMPLES OF MALE RATS TREATED ORALLY WITH PHOTOTHIDIAZURON- ^{14}C WHEN
 INCUBATED FOR 18 HOURS WITH ENZYMES AND ACIDS^a

Treatment	% Radioactivity					
	Organic Phase			Aqueous Phase		
	0-12	12-24	24-96	0-12	12-24	24-96
<u>beta</u> -Glucuronidase	8.5	15.2	12.7	91.5	84.8	87.3
Aryl Sulfatase	2.8	8.3	7.7	97.2	91.7	92.3
HCl ^b	30.5	22.7	17.4	69.5	77.3	82.6

^a Aqueous fractions from urine collected at 0-3 and 3-12 H were pooled to yield 0-12; 12-18 and 18-24 H were pooled to yield 12-24; and 24-48, 48-72, and 72-96 H were pooled to yield 24-96. Values are corrected for degradation in respective controls which averaged 7.8%.

^b For hydrolysis with HCl a final concentration of 9% (v/v) was used.

isolated from the urine of male rats treated orally with photothidiazuron- ^{14}C are given in Table VII. Major aglycones resulting from beta-glucuronidase cleavage included compounds V (4-hydroxyphenylurea), VIII, X, XII, and XIII (4-hydroxyphenylphotothidiazuron). Cleavage by aryl sulfatase yielded compounds XII and XIII (4-hydroxyphenylphotothidiazuron) as major aglycones. Acid cleavage yielded aglycones qualitatively similar to those produced by the enzymes (Table VII).

Fractionation of the feces showed that the highest level of photothidiazuron- ^{14}C equivalents in the organic phase was 13.6% at 12 hours; the lowest level was 4.8% at 24 hours. The highest level of radioactive material that partitioned into the aqueous phase was 24.9% at 24 hours. However, the majority of photothidiazuron- ^{14}C equivalents (>69%) remained in the fecal residue (Table VIII).

Tissue Residues Following Treatment of Rats with Photothidiazuron- ^{14}C

Concentration of radioactive material in the tissues of rats after 96 hours was low and in the parts per billion range (Table IX). Liver (14.7 ppb) and blood (10.7 ppb) contained the highest range of radioactive material; lung (6.3 ppb), kidney (4.5 ppb) peritoneal fat (4.9 ppb) and omental fat (4.0 ppb) were intermediate; and other tissues contained less than 4.0 ppb of radioactive material (Table IX).

IN VITRO STUDIES OF PHOTOTHIDIAZURON

Degradation of Photothidiazuron- ^{14}C by Rat Liver Subcellular Fractions

Table X shows the distribution of radioactive compounds following degradation of photothidiazuron- ^{14}C by various rat liver subcellular fractions. The percentage of radioactivity in the water fraction as

TABLE VII

NATURE AND CONCENTRATION OF THE RELEASED AGLYCONE MOIETY RESULTING FROM ENZYMATIC AND ACIDIC CLEAVAGE OF RADIOACTIVE WATER-SOLUBLE COMPOUNDS ISOLATED FROM URINE OF MALE RATS TREATED ORALLY WITH PHOTOTHIDIAZURON- $^{14}\text{C}^{\text{a}}$

Aglycone	% Aglycone Released Following Cleavage By ^b								
	β -Glucuronidase			Aryl Sulfatase			HCl		
	0-12	12-24	24-96	0-12	12-24	24-96	0-12	12-24	24-96
Origin	1.3	1.1	1.0	0.1	0.3	0.3	3.6	3.8	1.1
I	0.2	0.3	0.3	0.1	0.1	<0.1	1.0	1.2	0.5
III	0.1	0.4	<0.1	0.1	0.1	0.2	0.6	0.5	<0.1
IV	<0.1	2.0	<0.1	<0.1	0.1	<0.1	0.4	0.6	<0.1
V	0.6	1.4	1.2	0.2	0.6	0.7	2.8	4.4	3.2
VIII	0.9	1.3	1.7	0.1	0.4	0.4	3.3	4.1	1.8
X	1.7	1.5	3.0	0.3	0.5	1.1	6.5	2.5	3.6
XII	1.5	1.3	2.7	1.3	2.0	2.2	4.9	2.6	3.6
XIII	2.2	5.9	2.8	0.6	4.2	2.8	7.4	2.9	3.6

^aCompounds were separated by two dimensional TLC on glass plates coated with a 500- μ layer of silica gel GF₂₅₄; the solvent systems were ethyl acetate (first direction) and chloroform-ethyl acetate (1:1) (second direction). Compounds V, VIII, and XIII cochromatographed with 4-hydroxyphenylurea, 4-hydroxyacetanilide, and 4-hydroxyphenylphotothidiazuron, respectively.

^bValues are corrected for degradation in respective controls. Data from three rats.

TABLE VIII

RESULTS OF FRACTIONATION OF FECES FROM MALE RATS TREATED
ORALLY WITH PHOTOTHIDIAZURON- ^{14}C ^a

Time Interval Following Treatment (H)	% Radioactivity		
	Organic Phase	Aqueous Phase	Residue
0-12	13.6 (6.7)	17.2 (6.4)	69.0 (2.3)
12-24	4.8 (1.9)	24.9 (4.8)	70.3 (5.6)
24-96	6.7 (5.7)	14.2 (3.4)	79.1 (8.5)

^aData are means from at least 3 rats per time interval. Standard deviation is given in parentheses.

TABLE IX
CONCENTRATION OF RADIOACTIVE MATERIAL IN THE TISSUES
OF MALE RATS 96 HOURS AFTER ORAL TREATMENT WITH
PHOTOTHIDIAZURON- ^{14}Ca

Tissue	Ppb
Blood	10.7 (3.6)
Brain	1.2 (0.3)
Fat, Omental	4.0 (2.9)
Fat, Peritoneal	4.9 (3.1)
Heart	3.6 (2.0)
Intestine	2.8 (1.5)
Kidney	4.5 (1.3)
Liver	14.7 (3.6)
Lung	6.3 (2.5)
Muscle, Abdominal	3.1 (0.8)
Muscle, Back	2.3 (1.1)
Muscle, Leg	2.3 (1.1)
Pancreas	3.2 (1.4)
Spleen	3.2 (1.3)
Stomach	3.5 (1.1)
Testes	1.4 (0.8)

^aData are means of tissues from at least 3 rats.
Standard deviation is given in parentheses.

TABLE X
DISTRIBUTION OF RADIOCARBON-CONTAINING COMPOUNDS
FOLLOWING DEGRADATION OF PHOTOTHIDIAZURON- ^{14}C BY RAT
LIVER SUBCELLULAR FRACTIONS^a

Fraction	% Radioactivity	
	Organosoluble	Water Soluble
Whole Homogenate		
Alone	60.9	39.1
+ NADPH	26.4	73.6
Nuclear		
Alone	92.2	7.8
+ NADPH	90.1	9.9
Mitochondrial		
Alone	84.3	15.7
+ NADPH	79.8	20.2
Microsomal		
Alone	59.1	40.9
+ NADPH	52.0	48.0
Soluble		
Alone	76.8	23.2
+ NADPH	71.0	29.0
Control		
Alone	98.7	1.3
+ NADPH	98.1	1.9

^a Photothidiazuron- ^{14}C ($\sim 200,000$ dpm's) was incubated with indicated subcellular fraction with and without NADPH (2 μmoles) for 1 H at 38°C.

compared to that in the ethyl acetate fraction can be used as an index of the amount of degradation. The microsomal fraction was the most active subcellular unit yielding 40.9% water soluble alone and 48.0% water soluble + NADPH. The soluble and mitochondrial fractions also showed some degradative capacity, but the nuclear fraction was apparently inactive. The presence or absence of exogenous NADPH markedly influenced the degradative capacity of the whole homogenate. Alone, the whole homogenate yielded 39.1% of water soluble radioactive material, but in the presence of NADPH it yielded 73.6% (Table X).

Table XI gives the nature and relative concentration of organo-soluble radiocarbon-containing compounds following degradation of photothidiazuron- ^{14}C by the microsomal and soluble fractions. Clearly the major component was compound XV (photothidiazuron) accounting for greater than 90% of the activity. Lower levels of compound XIII (4-hydroxyphenylphotothidiazuron) were present in the microsomal fraction in the presence of NADPH. Compounds XVI and XVII found in this experiment were not detected in the in vivo studies.

Degradation of Photothidiazuron- ^{14}C by a Model Hydroxylating System

Incubation of photothidiazuron- ^{14}C in a model hydroxylating system for 6 hours yielded upon extraction 71.3% of the radioactive material in the aqueous fraction and 38.7% in the ethyl acetate fraction (Table XII). TLC of the ethyl acetate fraction indicated seven compounds plus material at the origin. Compounds I, IV, X, XII, XIII (4-hydroxyphenylphotothidiazuron), XV (photothidiazuron), and XVII were present. The relative concentrations of these compounds was consistently near 4.0%.

TABLE XI

NATURE AND RELATIVE CONCENTRATION OF ORGANOSOLUBLE RADIOCARBON-CONTAINING COMPOUNDS FOLLOWING DEGRADATION OF PHOTOTHIDIAZURON- ^{14}C BY RAT LIVER SUBCELLULAR FRACTIONS^a

Nature of Radioactive Material	% Radioactivity in Indicated Fraction				
	Control		Microsomal		Soluble
	Alone	+ NADPH	Alone	+ NADPH	+ NADPH
Origin	0.1	0.1	0.2	1.6	0.3
I	<0.1	<0.1	0.1	0.5	0.2
III	<0.1	<0.1	<0.1	0.1	0.1
V	0.2	0.1	0.1	0.4	0.1
XII	<0.1	<0.1	<0.1	0.5	<0.1
XIII	<0.1	0.1	0.2	4.8	1.5
XV	99.4	99.1	99.3	90.6	97.5
XVI	<0.1	<0.1	<0.1	1.2	<0.1
XVII	0.1	0.1	0.1	0.3	0.2

^a Photothidiazuron- ^{14}C (~200,000 dpm's) was incubated with indicated subcellular fraction with and without NADPH (2 μmoles) for 1 h at 38°C. Compounds were separated by two-dimensional TLC on glass plates coated with a 500- μ layer of silica gel GF₂₅₄; the solvent systems were ethyl acetate (first direction) and chloroform-ethyl acetate (1:1) (second direction). Compounds V, XIII, and XV cochromatographed with 4-hydroxyphenylurea, 4-hydroxyphenylphotothidiazuron, and photothidiazuron, respectively.

TABLE XII

NATURE AND RELATIVE CONCENTRATION OF RADIOCARBON-CONTAINING
COMPOUNDS FOLLOWING DEGRADATION OF PHOTOTHIDIAZURON- ^{14}C
BY A MODEL HYDROXYLATING SYSTEM^a

Nature of Radioactive Material	% Radioactivity ^b
Ethyl acetate soluble ^c	
Origin	2.8
I	4.1
IV	4.0
X	2.6
XII	4.0
XIII	3.2
XV	3.8
XVII	4.2
Water soluble	71.3

^a Photothidiazuron- ^{14}C ($\sim 200,000$ dpm's) was incubated in model hydroxylating system for 6 H at 38°C .

^b The control, which consisted of photothidiazuron and buffer gave 2.3% in the water and 97.7% in the ethyl acetate of which 99.8% was parent compound.

^c Compounds were separated by two-dimensional TLC on glass plates coated with a $500\text{-}\mu$ layer of silica gel GF₂₅₄; the solvent systems were ethyl acetate (first direction) and chloroform-ethyl acetate (1:1) (second direction). Compounds XIII and XV cochromatographed with 4-hydroxyphenyl-photothidiazuron and photothidiazuron, respectively.

CHAPTER V

DISCUSSION

Male rats treated with a single oral dose of photothidiazuron-aniline- ^{14}C at a level of 0.24 mg/kg rapidly absorbed and eliminated the compound primarily in the urine and to a lesser extent in the feces. By 96 hours posttreatment 75.5% of the administered dose was eliminated in the urine and 15.2% in the feces. This pattern of elimination differed from that reported previously for thidiazuron. By 96 hours male rats treated with a single oral dose of thidiazuron-aniline- ^{14}C at a level of 0.30 mg/kg eliminated 49.7 and 45.2% of the dose in the urine and feces, respectively; male rats treated with a single oral dose of thidiazuron-thiadiazole- ^{14}C at a level of 0.34 mg/kg eliminated 35.7 and 55.9% in the urine and feces; and female rats similarly treated with thidiazuron-thiadiazole- ^{14}C eliminated 40.8 and 50.8% in the urine and feces (Crecelius and Knowles, 1978). Moreover, female rats treated with either radiolabeled sample of thidiazuron (thiadiazole- ^{14}C or aniline- ^{14}C) as a dietary supplement at a concentration of 6.6 ppm for six days eliminated more radioactive material in the feces than in the urine; at the plateau stage rats eliminated about 90% of the daily dose of which 35% was in the urine and 55% was in the feces (Crecelius and Knowles, 1978). Thus it seems probable that the differences observed in the elimination patterns are a consequence of the structural differences between the two compounds.

Photothidiazuron metabolism by rats also was rapid, as judged by the partitioning behavior of the radioactive compounds present in the urine. During the 96 hour experimental period about 10% of the urinary radioactive material was ethyl acetate soluble and about 90% was water soluble. With thidiazuron about 20% was ethyl acetate soluble and about 80% was water soluble (Crecelius and Knowles, 1978).

The organosoluble photothidiazuron metabolites identified in rat urine included phenylurea, 4-hydroxyphenylurea, and 4-hydroxyphenylphotothidiazuron; identification of 4-hydroxyacetanilide should be regarded as tentative because of its low concentration. Some parent compound also was present. Cleavage data with beta-glucuronidase and aryl sulfatase indicated that 4-hydroxyphenylurea and 4-hydroxyphenylphotothidiazuron also were conjugated as glucuronides and sulfates. Figure 4 gives the metabolic paths for photothidiazuron based on this study.

The metabolic fate of thidiazuron in rats generally was qualitatively similar to that of photothidiazuron. Thidiazuron was converted to phenylurea and to 4-hydroxyphenylthidiazuron; the latter compound was conjugated with glucuronic and sulfuric acids (Crecelius and Knowles, 1978). Although an authentic sample of 4-hydroxyphenylurea was not available for comparison with thidiazuron metabolites at the time that study was conducted, it seems likely that 4-hydroxyphenylurea was not present at least in the free form. This contention is supported by the fact that the only thidiazuron metabolite reported by Crecelius and Knowles (1978) that contained only the aniline moiety radiolabel was phenylurea.

The actual mechanism for the formation of phenylurea remains obscure. It was not formed from photothidiazuron in vitro by the model hydroxylating system (which can form products in addition to hydroxylated

Figure 4. Paths for photothidiazuron metabolism by rats.

derivatives) or by the microsomal and soluble fractions from rat liver. Also it was not formed from thidiazuron under similar conditions (Crecelius and Knowles, 1978). The in vitro experiments with rat liver subcellular fractions mentioned above used a relatively short incubation time of one hour. There is now evidence that phenylurea does form in vitro from certain rat liver subcellular fractions but that a longer incubation time is required (Benezet and Knowles, 1978). This suggests that an intermediate may be involved in the formation of phenylurea.

The formation of 4-hydroxyphenylurea and of its glucuronide and sulfate conjugates in rats is consistent with metabolism studies of phenylurea in rabbits reported previously (Bray et al., 1949). Analysis of rabbit urine indicated that an average of 46% of an oral dose was hydroxylated to yield 4-hydroxyphenylurea which was subsequently conjugated with glucuronic acid (26%) and sulfuric acid (20%). Some free 4-hydroxyphenylurea also was present (Bray et al., 1949).

CHAPTER VI

SUMMARY

Photothidiazuron is a major photoconversion product of thidiazuron defoliant on cotton leaves. The purpose of the present study was to investigate its excretion balance, metabolic fate, and tissue residues following oral administration to rats. Studies of this type with pesticide degradation products aid in the assessment of potential hazards that might be associated with commercial use of the parent pesticide.

The results of these experiments are summarized below:

1. Photothidiazuron, radiolabeled in the aniline moiety, was rapidly absorbed and eliminated following oral administration to rats. By 96 hours posttreatment, 90.7% of the administered dose was accounted for in the urine (75.5%) and feces (15.2%).

2. Between 5.0 and 10% of the radioactive material in the urine partitioned into ethyl acetate. In addition to photothidiazuron and several unknowns, the following metabolites were present: 4-hydroxyphenylphotothidiazuron, phenylurea, and 4-hydroxyphenylurea. There also was some evidence for the presence of 4-hydroxyacetanilide.

3. The major portion of the radioactivity (>90%) remained in the urine after extraction with ethyl acetate. Cleavage data with beta-glucuronidase and aryl sulfatase indicated that at least 12.1% and 6.3%, respectively, of the urinary radioactive materials were conjugated with glucuronic and sulfuric acids. Aglycones included 4-hydroxyphenylurea, 4-hydroxyphenylphotothidiazuron, and several unknowns.

4. Fractionation of the feces indicated that about 10% of the radioactive material was organosoluble, about 20% was water soluble and about 70% was unextractable from the fecal residue.

5. Tissue levels of radioactivity were low and in the ppb range.

6. There is no evidence from these excretion balance, metabolic fate, and tissue residues studies of photothidiazuron in the rat that its formation on plant leaf surfaces would pose any toxicological hazards.

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The undersigned, appointed by the Dean of the Graduate Faculty, have examined a thesis entitled

EXCRETION BALANCE, METABOLIC FATE, AND TISSUE RESIDUES OF
N-PHENYL-N'-1,2,5-THIADIAZOL-3-YLUREA (PHOTOTHIDIAZURON)
IN RATS

presented by

TERRY L. JOHNSON

a candidate for the degree of

MASTER OF SCIENCE

and hereby certify that in their opinion it is worthy of acceptance.

Charles Knowles

Thomas R. Yonke

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