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Metabolism of Trifluralin, Profluralin, and Fluchloralin by Rat Liver Microsomes[1]

J. O. NELSON,* P. C. KEARNEY,† J. R. PLIMMER,† AND R. E. MENZER*

*Department of Entomology, University of Maryland, College Park, Maryland 20742; and †Agricultural Environmental Quality Institute, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland 20705

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Three structurally related [^{14}C]dinitroaniline herbicides, trifluralin, profluralin, and fluchloralin, were extensively metabolized *in vitro* by both normal and phenobarbital-induced rat liver microsomes. Identification of the metabolites in the ethyl acetate extracts indicated that aliphatic hydroxylation, *N*-dealkylation, reduction of a nitro group, and cyclization were the predominant metabolic routes for these herbicides *in vitro*. Of particular interest was the formation of a benzimidazole metabolite.

INTRODUCTION

Several substituted dinitroaniline herbicides are currently being marketed. Although some work has been published on their metabolism in plants, soil, and microorganisms as well as their photochemistry, relatively little is known of the mammalian metabolism of these herbicides (1). The existing literature on mammalian metabolism deals with two of the earliest compounds, α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine (trifluralin) and α,α,α-trifluoro-2,6-dinitro-*N*-butyl-*N*-ethyl-*p*-to-luidine (benefin). The metabolism of trifluralin was reported for rats, dogs (2), and ruminants (3). We now report the *in vitro* metabolism of trifluralin, *N*-(2-chloroethyl)-α,α,α-trifluoro-2,6-dinitro-*N*-propyl-*p*-toluidine (fluchloralin), and *N*-(cyclopropylmethyl)-α,α,α-trifluoro-2,6-dinitro-*N*-propyl-*p*-toluidine (profluralin) by rat hepatic microsomes.

MATERIALS AND METHODS

The structures of the three dinitroaniline herbicides are found in Table 1. The specific activity in microCuries per milligram, position of the ^{14}C, and the source of each herbicide are as follows: trifluralin (TF),[2] 9.0, CF_3, Eli Lilly, Greenfield, Indiana; profluralin (PF), 34.3, ring, CIBA-Geigy Corp., Greensboro, North Carolina; fluchloralin (FL), 37.9, ring, BASF Wyandotte Corp., Parsippany, New

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[2] Abbreviations used: TF, trifluralin; PF, profluralin; FL, fluchloralin; tlc, thin-layer chromatography; G-6-P, glucose-6-phosphate; G-6-PD, glucose-6-phosphate dehydrogenase; ODS, octadecyltrimethyloxysilane; hplc, high performance liquid chromatography: GC/MS, gas chromatography/mass spectometry.

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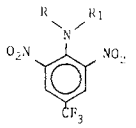
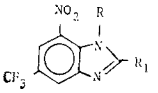
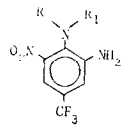
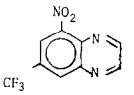
TABLE 1

Structures and Thin-Layer Chromatography (tlc) R_f Values of Reference Dinitroaniline Compounds and Metabolites Identified in Rat Liver Microsomal Studies

Compound	R	R_1	Metabolite present in organic extract			tlc R_f values of standard reference compounds	
			Trifluralin	Profluralin	Fluchloralin	Benzene	Benzene: Ethyl acetate (1:1)
							
Trifluralin (TF-1)	$-CH_2CH_2CH_3$	$-CH_2CH_2CH_3$	x[a]	x[b]	ND[a]	0.63	0.71
Profluralin (PF-1)	$-CH_2CH_2CH_3$	$-CH_2\triangleleft$	ND	x	ND	0.63	0.64
Fluchloralin (FL-1)	$-CH_2CH_2CH_3$	$-CH_2CH_2Cl$	ND	ND	x	0.61	0.65
TF-2	$-CH_2CH_2CH_3$	$-H$	x	x	x	0.59	0.71
PF-2	$-H$	$-CH_2\triangleleft$	ND	x	ND	0.58	0.64
FL2	$-H$	$-CH_2CH_2Cl$	ND	ND	ND	0.54	0.64
TF-3	$-H$	$-H$	x	x	x	0.43	0.68
TF-6	$-H$	$-CH_2CH_2CH_2OH$	x	x	x	0.02	0.52
TF-7	$-H$	$-CH_2CH(OH)CH_3$	x	x	x	0.05	0.63
TF-8[c]	$-CH_2CH_2CH_3$	$-CH_2CH_2CH_2OH$	x	x[b]	ND	NA[d]	NA
TF-9[c]	$-CH_2CH_2CH_3$	$-CH_2CH(OH)CH_3$	x	x[b]	ND	NA	NA
							
TF-4	$-CH_2CH_2CH_3$	$-CH_2CH_3$	ND	ND	ND	0.06	0.50
TF-5	$-H$	$-CH_2CH_3$	x	x	x	0.01	0.34
							
TF-14	$-H$	$-H$	x	ND	x	0.06	0.35
							
FL-6			ND	ND	x	0.13	0.50

[a] x means compound was detected by GC/MS analysis; ND means compound was not detected by GC/MS analysis.

[b] Trifluralin was a contaminant of profluralin at approximately 0.5% level and was in trace quantities along with TF-8 and TF-9 by GC/MS.

[c] Structure as determined by mass spectrometry.

[d] Reference compound not available.

Jersey. Each ^{14}C-labeled compound was purified by preparative thin-layer chromatography (tlc) on 0.25-mm silica gel 60-F254, using benzene as a solvent system. Standard reference compounds TF-1, TF-2, TF-3, TF-4, and TF-5; PF-1 and PF-2; and FL-1, FL-2, and FL-6 (Table 1) were supplied by Eli Lilly, CIBA–Geigy, and BASF Wyandotte, respectively.

Synthesis

Several reference metabolites, which were not available, were synthesized for comparison with isolated metabolites. 2,6-Dinitro-*N*-(*n*-propan-3-ol)-α,α,α-trifluoro-*p*-toluidine (TF-6) was synthesized by the dropwise addition of 4-chloro-3,5-dinitro-α,α,α-trifluorotoluene [0.015 mol, prepared by the method of Hall and Choo-Seng (4)] dissolved in benzene to a stirred solution of 3-amino-1-propanol (0.045 mol, Aldrich) in 25 ml of benzene at 26°C for 1 hr. The benzene was washed with water, dried with Na_2SO_4, and concentrated to yield 4.6 g of yellow crystalline TF-6: mp, 96–98°C; ir (KBr pellet), 3300–3600 (OH), 3350 (NH) cm^{-1}; nmr (in $CDCl_3$), δ 9.2 (*s*, NH), 8.4 (*s*, Ph), 3.9 (*t*, CH_2), 3.2 (*q*, CH_2), 2.0 (*m*, CH_2), and 1.9 (*s*, OH); mass spectrum, *m/e* 309 (parent), 264 (base).

2,6-Dinitro-*N*-(*n*-propan-2-ol)-α,α,α-trifluoro-*p*-toluidine (TF-7) was synthesized by the same procedure as TF-6, except 1-amino-2-propanol (Aldrich) was the reacting amine. The yellow crystalline product (4.4 g) has a mp of 64–66°C; ir, 3600 (OH), 3350 (NH) cm^{-1}; nmr, δ 9.2 (*s*, NH), 8.4 (*s*, Ph), 4.1 (*q*, CH), 3.1 (*t*, CH_2), 2.0 (*s*, OH) and 1.3 (*d*, CH_3); mass spectrum, *m/e* 264, 247, 160 (base), no parent ion. 2-Amino-6-nitro-α,α,α-trifluoro-*p*-toluidine (TF-14) was synthesized by the method of Leitis and Crosby (5).

Incubation

Hepatic microsomes from normal and phenobarbital-pretreated male Sprague–Dawley rats (126–150 g) were prepared as previously described (6). The rats were injected with 75 mg/kg of phenobarbital (ip) for three consecutive days before killing. Livers were homogenized and microsomes were prepared in 0.15 *M* KCl. The washed microsomal pellet was suspended in 0.1 *M* phosphate buffer, pH 7.4. Protein concentrations were determined by the Lowry method (7) and cytochrome *P*-450 concentrations by the method of Omura and Sato (8). Incubations were as follows: 4.0 mg of microsomal protein, 1.8 μmol of NADP, 18 μmol of glucose-6-phosphate (G-6-P), 0.4 Kornberg units of glucose-6-phosphate dehydrogenase (G-6-PD), and 10 μl of the [^{14}C]dinitroaniline (0.1 μCi) in methyl cellosolve were brought up to a total incubation volume of 6.0 ml with 0.1 *M* phosphate buffer, pH 7.4, in 25-ml Erlenmeyer flasks. Duplicate incubations were performed in the dark for 1 hr at 37°C with mild shaking. Heat inactivated microsomes (100°C for 10 min) and incubations without added cofactors served as controls.

Extraction and Cleanup

Incubation mixtures were extracted twice with equal volumes of ethyl acetate. The aqueous phase and precipitated microsomal solids were separated by Millipore filtration through 0.45-μm filters. The collected solids on the Millipore filters were solubilized with 1.0 ml of Soluene (Packard) in a counting vial for about 2 hr, after which scintillation solution was added and ^{14}C determined by liquid scintillation counting. Aliquots of the aqueous and organic phases taken immediately after completion of the separations were also counted and all samples were corrected to dpm using [^{14}C]hexadecane as an internal standard. The organic extracts were dried with sodium sulfate, stored in stoppered tubes until they were concentrated to a small volume (ca. 0.01 ml) and spotted on tlc plates which were then developed

TABLE 2

Distribution of [^{14}C]Dinitroaniline Herbicides and Their Metabolites after Incubation with Rat Liver Microsomes

Compound	Microsomes[b]	Percentage of added ^{14}C-radioactivity[a]											
		Complete[c]				Control I[d]				Control II[e]			
		Aqueous	Organic	Solids	Total	Aqueous	Organic	Solids	Total	Aqueous	Organic	Solids	Total
Trifluralin	Normal	3.8	65.2	14.3	83.3	0.2	81.1	5.6	83.3	0.1	86.0	3.0	89.1
	Induced	6.8	47.1	12.2	66.2	0.1	88.9	2.8	91.9	0.2	71.0	3.2	74.5
Profluralin	Normal	6.7	59.7	15.3	81.8	0.4	79.6	4.7	84.8	0.1	81.6	6.6	88.3
	Induced	4.6	36.8	17.6	58.9	0.6	87.1	5.2	93.0	0.2	85.5	2.6	88.3
Fluchloralin	Normal	10.3	63.7	13.7	87.7	1.0	78.0	16.9	95.9	0.3	103.3	2.4	106.0
	Induced	8.8	45.0	17.5	71.4	1.2	105.5	3.6	110.3	0.8	98.3	5.1	104.1

[a] Values are averages from two experiments in which duplicate incubation mixtures were used for the complete mixtures and single incubations for the controls. All three compounds were incubated with the same microsomal preparation in each experiment for comparative purposes.

[b] Microsomes were prepared from normal male Sprague–Dawley rats or, rats which had been pretreated with phenobarbital for 3 consecutive days (75 mg/kg, ip).

[c] The incubation mixture contained 4.0 mg of microsomal protein, 1.8 μmol of NADP, 18 μmol of G-6-P, 0.4 Kornberg Units of G-6-P dehydrogenase and 0.1 μCi of [^{14}C]dinitroaniline herbicide added in 10 μl methyl cellosolve, all in 6.0 ml of 0.1 *M* phosphate buffer, pH 7.4.

[d] Control I: same as above, except the microsomal fraction had been heat inactivated at 100°C for 10 min.

[e] Control II: same as complete, without the NADPH-generating system.

TABLE 3

Thin-layer Chromatographic Separation of Ethyl Acetate Extractable ^{14}C from Microsomal Incubation Mixtures with Dinitroaniline Herbicides

Compound	Microsomes	Percentage of ethyl acetate extractable ^{14}C		
		Nonpolar[a]	Intermediate Polarity[b]	Polar[c]
Trifluralin	Normal	63.5	27.1	9.4
	Induced	13.3	39.5	47.2
Profluralin	Normal	44.3	29.3	26.4
	Induced	43.4	19.9	36.7
Fluchloralin	Normal	51.9	22.3	25.8
	Induced	37.6	5.8	56.6

[a] Percentage of ^{14}C with $R_f > 0.2$. Solvent system, benzene.
[b] Remainder of ^{14}C not in benzene or benzene:ethyl acetate solvent system.
[c] Percentage of ^{14}C with $R_f < 0.2$. Solvent system, benzene:ethyl acetate (1:1).

with benzene or benzene:ethyl acetate (1:1). Radioactive metabolites were detected by autoradiography using "No-Screen" medical X-ray film. Preparative tlc was used for initial purification of organic extracts from mass incubations. Colored bands of silica gel were scraped from the plates and eluted with ethyl acetate. Total radioactivity in each band was determined by counting an aliquot. The tlc fractions were examined by using a DuPont Model 21-491B gas chromatograph/mass spectrometer interfaced with a Hewlett Packard Model 2100A computer. Mass spectra and chromatographic behavior were compared with known standards (Table 1). A DuPont 830 high pressure liquid chromatograph, fitted with a uv detector and a 1-m (2.1 mm i.d.) column packed with Permaphase ODS (octadecyltrimethyloxysilane), was used to compare retention times with authentic standards. Mixtures of methanol and water used as the mobile phase.

TABLE 4

Distribution of ^{14}C after Extractions of Multiple Incubations[a]

Compound	Percentage of added ^{14}C-radioactivity			
	Aqueous	Organic	Solids	Total
Trifluralin				
Complete[b]	2.1	88.3	5.3	95.9
Control I	0.5	91.3	3.4	95.2
Control II	1.3	59.6	16.4	77.2
Profluralin				
Complete	3.6	88.2	6.0	97.8
Control I	2.1	110.0	3.0	115.1
Control II	2.1	87.5	10.0	99.6
Fluchloralin				
Complete	1.4	83.3	0.7	85.4
Control I	1.3	78.6	4.0	83.9
Control II	1.4	56.2	21.7	79.3

[a] See Table 2 for general incubation conditions. Modification in procedure was a 100-fold increase in substrate concentration.
[b] All incubations employed microsomes from phenobarbital-pretreated rats.

RESULTS

Comparison of Metabolism of Trifluralin, Profluralin, and Fluchloralin by Normal and Phenobarbital-induced Microsomes

The distributions of ^{14}C-radioactivity following incubation and extraction procedures are shown in Table 2. The presence of polar metabolites for all three compounds was indicated by the radioactivity (4–10%) in the aqueous phase, which is not found in the controls. The effect of induction on the amount of aqueous soluble products was variable. Simular results were observed in the solid's "bound" radioactivity, except this fraction accounted for higher percentages of the total activity.

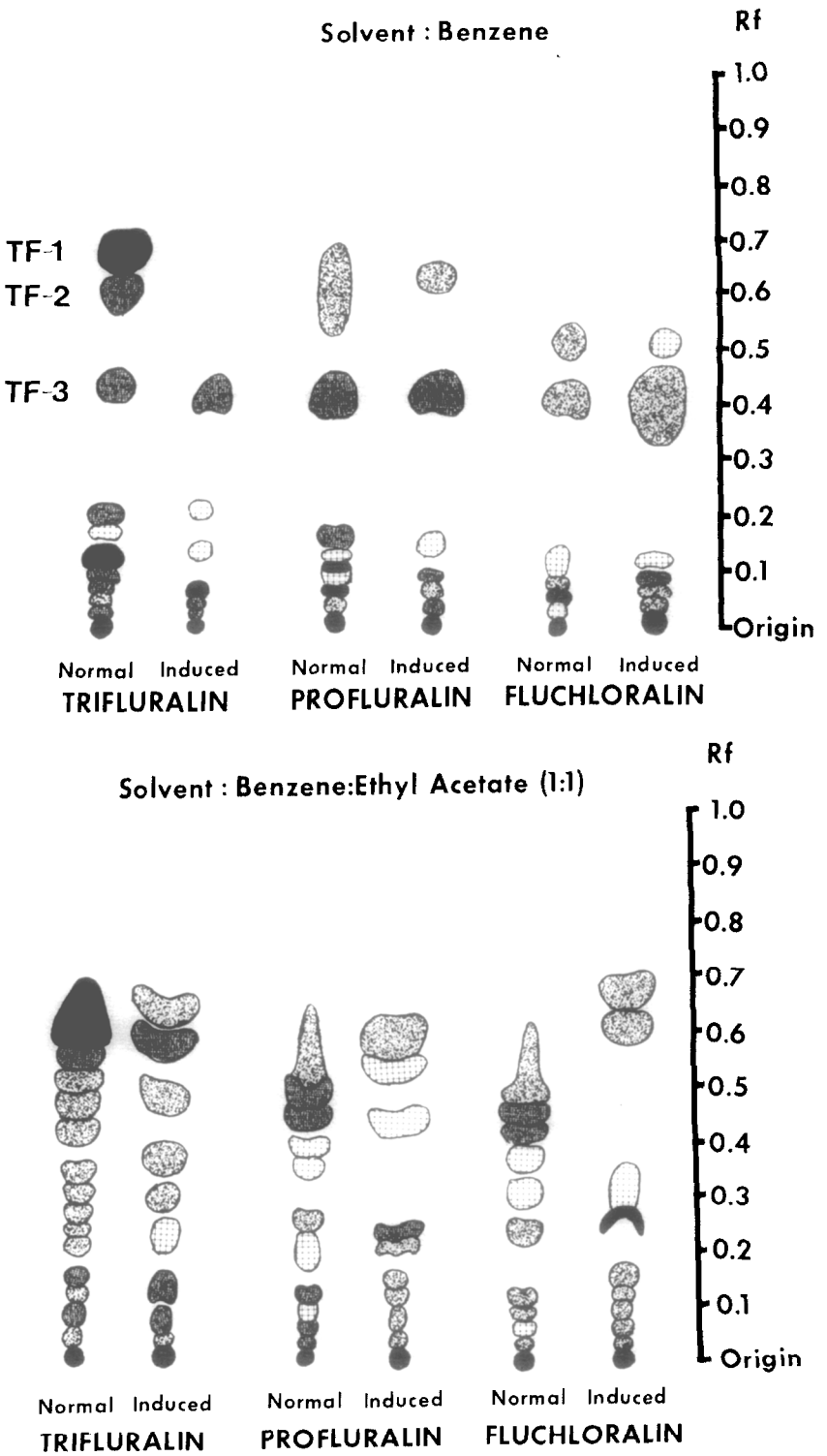


FIG. 1. *Representations of autoradiograms from the tlc separations of ethyl acetate extractable metabolites from rat-liver microsomal incubations with* [^{14}C]*dinitroaniline herbicides. Increased shading indicates an increased amount of radioactivity.*

The effect of induction was consistent only in the organic extractable radioactivity. The percentage of ^{14}C recovered in the organic extract of induced microsomal incubations was always lower than those recovered from the normal microsomes. This lower value for the induced system was also reflected in lower total recovery of added ^{14}C-radioactivity. There were no distinct differences in radioactivity distribution patterns among the three compounds.

Examination of the radioactivity of the ethyl acetate extract by tlc and autoradiography is represented in Fig. 1. In the benzene solvent system, nonpolar compounds TF-1, TF-2, and TF-3 were separated by tlc (R_f 0.3 to 0.8). For tables of tlc R_f values, hplc retention times, and glc retention times see Kearney *et al.* (9). A principal metabolite from each incubation was the dealkylated aniline (TF-3, $R_f = 0.43$). A quantitative estimate of the radioactivity present in each spot, based upon scraping and counting the silica gel, indicated that consistently more radioactivity was in the polar fraction ($R_f < 0.2$) when incubations employed induced microsomes. Many of the polar metabolites in the benzene system were separated between R_f 0.2 and 0.6 in the benzene:ethyl acetate (1:1) solvent system. In benzene:ethyl acetate, the nonpolar metabolites (TF-2, PF-2, FL-2, and TF-3) were unresolved (R_f 0.6–0.7). Again, there was a group of polar products with $R_f < 0.2$. A third grouping of compounds of intermediate polarity occurred below $R_f = 0.2$ in the benzene system and above $R_f = 0.2$ in the benzene:ethyl acetate (1:1) system. This group of compounds includes TF-4, TF-5, TF-6, TF-7, TF-14, and FL-6. The tlc separation of ^{14}C-radioactivity from the organic extracts into these three arbitrary classes is summarized in Table 3. While no distinct differences between compounds were seen in the distribution of ^{14}C-radioactivity during the extraction phase (Table 2), Table 3 shows there are indeed differences when the ethyl acetate extractable metabolites are examined by tlc. Induction drastically reduces the amount of nonpolar products for trifluralin, has little or no effect on profluralin, and slightly reduced nonpolar metabolites of fluchloralin. When induced microsomes were used, the metabolites of intermediate polarity increased with induction for trifluralin, but decreased for both profluralin and fluchloralin. The one consistent change was an increase in polar products for all three herbicides when incubation employed induced microsomal preparations.

Multiple Incubations for Identification of Metabolites

Microsomal preparations from induced rats were used for multiple incubations. The concentration of each herbicide was increased 100-fold to provide larger quantities of metabolites for identification purposes. A summary of the distribution of radioactivity after incubations and extractions is seen in Table 4. A comparison of these values with the values in Table 2 for induced microsomes shows a decrease in aqueous phase and solids "bound" radioactivity and an increase in the ethyl acetate extractable products, which was expected with higher substrate concentrations. The organic extracts were subjected to preparative tlc (benzene:ethyl acetate, 1:1), and the metabolite bands of silica gel were scraped, eluted with ethyl acetate, and concentrated. These extracts were used directly for GC/MS identification and cochromatography with standards on tlc and hplc.

A summary of the metabolites identified is found in Table 1. Among the metabolites identified were *N*-dealkylation products TF-2, PF-2, and TF-3; aliphatic hydroxylation products TF-8 and/or TF-9; products of both *N*-dealkylation and aliphatic hydroxylation, TF-6 and TF-7; a product of nitro reduction TF-14; and cyclized

TABLE 5

Mass Spectral Data

Compound	*m/e* (%)
TF-1	335 (12.6), 307 (13.8), 306 (100.0), 290 (11.9), 264 (61.7)
TF-2	293 (17.9), 264 (100.0), 248 (22.8), 207 (22.4), 177 (11.8), 160 (12.2), 159 (12.5)
TF-3	251 (100.0), 189 (13.3), 159 (29.3)
TF-5	259 (100.0), 258 (85.4), 213 (27.5), 212 (26.6)
TF-6	309 (28.3), 190 (19.6), 274 (21.6), 264 (100.0), 248 (19.5), 245 (13.6), 244 (33.7), 235 (36.6), 228 (21.3), 217 (13.6), 206 (11.0), 202 (13.2), 201 (10.2), 200 (11.1), 199 (11.8), 198 (12.7), 189 (11.4), 188 (11.1), 187 (14.7), 186 (11.2)
TF-7	278 (7.6), 264 (5.7), 248 (7.6), 247 (33.0), 235 (11.6), 220 (29.3), 218 (15.8), 217 (84.7), 206 (12.6), 202 (25.7), 201 (39.2), 190 (14.3), 189 (38.9), 188 (37.1), 187 (82.0), 186 (16.8), 174 (43.9), 173 (62.0), 172 (59.2), 171 (22.4), 160 (100.0)
TF-8 or TF-9[a]	351 (5.1), 307 (14.5), 306 (100.0), 290 (7.2), 264 (51.2), 248 (23.2)
TF-14	221 (100.0), 204 (10.8), 203 (26.0), 175 (21.6), 174 (19.3), 173 (11.8), 166 (11.7), 158 (12.1), 148 (15.2)
PF-1	347 (3.8), 330 (8.2), 318 (12.2), 264 (3.0), 69 (12.3), 55 (100.0)
FL-1	357 (5.5), 355 (11.0), 340 (2.0), 338 (7.2), 336 (2.4), 328 (30.9), 326 (74.7), 310 (12.1), 306 (100.0), 264 (39.5), 248 (18.1), 65 (15.0), 63 (61.3)
FL-2	315 (6.0), 313 (14.8), 264 (100.0), 218 (11.2), 217 (9.5), 206 (11.5), 186 (13.5), 172 (16.6), 160 (25.6)
FL-6	243 (79.0), 224 (5.8), 213 (3.1), 198 (10.0), 197 (100.0), 185 (36.1), 170 (19.9), 143 (30.3)

[a] The position of hydroxylation on the *N*-propyl group was not determined.

metabolites TF-5 and FL-6. Mass spectrometry was used for determination of molecular weight and gave valuable structural information (Table 5). A principal fragmentation pathway observed was the loss of an alkyl radical from the *N*-alkyl group to form a stable ion in which the positive charge was localized on the anilino-nitrogen atom. Examples of this fragmentation include: TF-1, *m/e* 306 (M-29); TF-2, *m/e* 264 (M-29); TF-6, *m/e* 264 (M-45); and FL-1, *m/e* 306 (M-49). The principal fragments for the benzimidazole metabolite were M-1, M-46, and M-47 which correspond to the loss of H and/or NO_2. A manuscript discussing the mass spec-

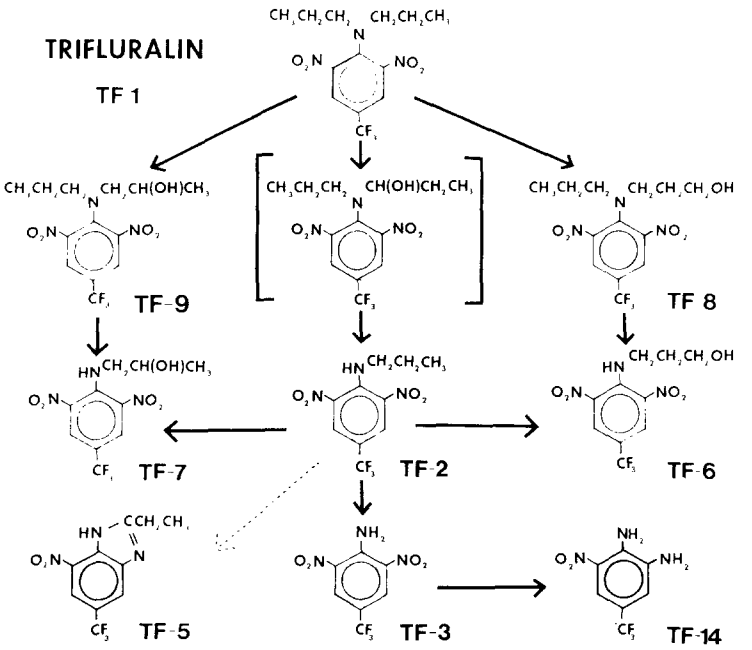


FIG. 2. *Proposed metabolic pathway for trifluralin by rat liver microsomes.*

trometry of dinitroaniline herbicides and related compounds is in preparation.

DISCUSSION

The products identified in this study (Table 1) suggest metabolic pathways for trifluralin shown in Fig. 2. Principal metabolites were the two monodealkylated alcohols TF-6 and TF-7 and the aniline TF-3. The pathways seem to indicate hydroxylation of the *N*-propyl group at each of the three carbons, since oxidation of the α carbon to an unstable α hydroxy intermediate is the presumed intermediate in *N*-dealkylation. Of the two stable alcohols, the secondary alcohol TF-7 predominated. A similar effect has been observed in microsomal oxidation of fatty acids and hydrocarbons where the penultimate carbon (ω-1) was the predominating site of attack beyond a certain carbon chain length (10, 11). The toxicological importance of these alcohols is unknown, but they are probably intermediates in the formation of more polar products such as acids and possibly cyclization products.

The product, TF-14, formed by reduction of a nitro group was identified by GC/MS but was present in only minute amounts. Reduction is a much more important pathway *in vivo* (2) than is indicated by our study of aerobic *in vitro* metabolism. The intermediate nitroso and hydroxylamino derivatives, formed in the sequential reduction of nitro groups by cytochrome P-450 or NADPH-cytochrome C reductase, may be autoxidized to nitro groups under aerobic conditions, thus little or no nitro reduction products would be expected (12). Presumably under anaerobic conditions such reduction products would predominate. This metabolite could also react with sugars, acids, and ketones (5) and thus escape detection as the 1,2-phenylenediamine.

The other metabolite identified from the organic extract was the benzimidazole TF-5. This is the first report of a benzimidazole as a metabolite of dinitroaniline herbicides in a mammalian system. Benzimidazoles have previously been reported as soil metabolites (9) and also as photolysis products of trifluralin (5). The former studies also identified the *N*-alkylated benzimidazole TF-4 as a metabolite and photolysis product of trifluralin, but we did not detect this compound. The mechanism by which benzimidazoles form in this *in vitro* system is unknown.

The metabolites of profluralin included the *N*-dealkylation products TF-2, PF-2, and TF-3; the alcohols TF-6 and TF-7, and the benzimidazole TF-5.

Fluchloralin gave similar metabolites to profluralin and trifluralin (Table 1) but fluchloralin metabolites also included the unusual quinoxaline compound, FL-6. This cyclization product is also found as a soil metabolite (9) and photolysis product (13) of fluchloralin and presumably arises from cyclization between the 2-chloroethyl group and a nitro group.

In general, the predominant metabolic pathways for the three dinitroaniline herbicides in this system appeared to be aliphatic hydroxylation or *N*-dealkylation. To a lesser extent, each compound cyclized to form a benzimidazole and also a quinoxaline in the case of fluchloralin. Reduction of the nitro group to an amine was potentially an important metabolic pathway, but was not observed under the conditions employed in this work.

The toxicological significance of the metabolites is unknown, but the possible mutagenic effects of metabolites containing a benzimidazole system should be investigated in light of the mutagenicity of benzimidazole in a bacterial test system (14). A structure–activity study of various benzimidazoles indicated that 2-ethyl benzimidazole was the most active of a series of 2-substituted compounds (15). Substitution of a nitro group in the 4(7) position of benzimidazole also increased mutagen-

icity. The benzimidazole metabolite TF-5 produced by all three herbicides has an ethyl group in the 2-position, a nitro group in the 4(7)-position, and a trifluoromethyl group in the 6(5) position. The structural similarity of benzimidazole metabolites to known mutagens and natural nucleobases meet two of the four criteria outlined by Seiler (16) for pesticides which should be tested for mutagenic potential.

The effect of phenobarbital induction on the microsomal metabolism of the three dinitroaniline herbicides is an increase in the proportion of polar products formed. This not unexpected effect is seen most readily in the tlc behavior of the nonpolar and polar metabolites, where the radioactivity of the polar fraction consistently increases with induction. The distribution data, in particular the aqueous and solid fractions (Table 2), and tlc behavior of the nonpolar and intermediate polarity products (Table 3), vary with the compound and are not strictly a function of induction. The significance of induction to the metabolism of these dinitroaniline herbicides will be uncertain until the polar metabolites can be identified.

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