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Metabolism in Human Embryonic Lung Cell Cultures of Three Phenylurea Herbicides: Chlorotoluron, Fluometuron, and Metobromuron

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Three phenylurea herbicides, chlorotoluron [*N*-(3-chloro-4-methylphenyl)-*N'*,*N'*-dimethylurea], fluometuron [*N*-(3-trifluoromethylphenyl)-*N'*,*N'*-dimethylurea], and metobromuron [*N*-(4-bromophenyl)-*N'*-methoxy-*N'*-methylurea], were found to be very resistant to the metabolic action of human embryonic lung (HEL) cells in culture. Over 95% of the recovered radioactivities from these ^{14}C-labeled compounds after incubation with HEL cell cultures remained intact, with less than 3 and 2% of the remainder being organoextractable and water-soluble metabolites, respectively. Oxidative metabolism predominated over hydrolytic metabolism for all three compounds. Oxidative metabolites were identified by cochromatography as follows—for chlorotoluron: *N*-(3-chloro-4-methylphenyl)-*N'*-formyl-*N'*-methylurea, *N*-(3-chloro-4-methylphenyl)-*N'*-formylurea, *N*-(3-chloro-4-methylphenyl)-*N'*-methylurea, and *N*-(3-chloro-4-methylphenyl)urea; for fluometuron: *N*-(3-trifluoromethylphenyl)-*N'*-formyl-*N'*-methylurea, *N*-(3-trifluoromethylphenyl)-*N'*-methylurea, and *N*-(3-trifluoromethylphenyl)urea; and metobromuron: *N*-(4-bromophenyl)-*N'*-methylurea, *N*-(4-bromophenyl)urea, and *N*-(4-bromophenyl)-*N'*-hydroxy-*N'*-methylurea. The amounts of water-soluble metabolites of these compounds were too low for identification.

Cell culture systems provide a very useful method for investigating the direct actions of xenobiotics on cells and tissues in the absence of the complex system in a whole organism (Rosenoer, 1966). Cultured mammalian cells have been used to study metabolism of pesticides. Baron and Locke (1970) reported carbaryl metabolism by a human embryonic lung cell line. North and Menzer (1970) studied growth and esterase inhibition in cultured L-929 mouse fibroblast cells for four organophosphorus insecticides. They also reported biotransformation of dimethoate (North and Menzer, 1972), DDT (North and Menzer, 1973), carbaryl (Lin et al., 1975a), and chlordimeform (Lin et al., 1975b) in primary human embryonic lung cell cultures.

Chlorotoluron, fluometuron, and metobromuron are three phenylurea herbicides. Only a few studies have been reported on the metabolism of these compounds. Mücke et al. (1976) administered [^{14}C]chlorotoluron to rats and found that chlorotoluron was demethylated to *N*-(3-chloro-4-methylphenyl)-*N'*-methylurea and *N*-(3-chloro-4-methylphenyl)urea. It was then further oxidized to form benzylic alcohol derivatives and benzoic acid derivatives. The benzyl alcohol derivative was demethylated to *N*-(3-chloro-4-hydroxymethylphenyl)-*N'*-methylurea. The benzoic acid was subsequently demethylated to *N*-(3-chloro-4-carboxyphenyl)-*N'*-methylurea and *N*-(3-chloro-4-carboxyphenyl)urea. The benzylic alcohol derivatives could also be conjugated. In vitro metabolism of chlorotoluron in rats and Japanese quail has been reported by Hinderer and Menzer (1976a,b).

The metabolism of fluometuron has been investigated mainly in plants. In cotton and cucumber, fluometuron was metabolized to form demethylfluometuron, *N*-(3-trifluoromethylphenyl)urea, and 3-(trifluoromethyl)aniline (Rogers and Funderburg, 1968). The metabolic fate of fluometuron in animals as well as in tissue or cell cultures is still unknown.

The metabolism of metobromuron in plants and animals has been investigated by Geissbühler and Voss (1972). Three possible metabolic pathways were reported: (1) metobromuron undergoes metabolic change to form in sequence, *N*-(4-bromophenyl)-*N'*-methoxyurea, *N*-(4-bromophenyl)-*N'*-hydroxyurea, and *N*-(4-bromophenyl)-urea; (2) metobromuron forms *N*-(4-bromophenyl)-*N'*-hydroxy-*N'*-methylurea, *N*-(4-bromophenyl)-*N'*-hydroxyurea, and *N*-(4-bromophenyl)urea; (3) metobromuron becomes *N*-(4-bromophenyl)-*N'*-hydroxy-*N'*-methylurea, *N*-(4-bromophenyl)-*N'*-methylurea, and *N*-(4-bromophenyl)urea.

The results of investigations on the metabolism of these three phenylurea compounds in primary human embryonic lung cells in culture are reported herein.

MATERIALS AND METHODS

Chemicals. [^{14}C]Chlorotoluron (sp act. 3.77 mCi/mmol) and [^{14}C]fluometuron (sp act. 2.42 mCi/mmol) were labeled on the phenylmethyl groups. [^{14}C]Metobromuron (sp act. 2.1 mCi/mmol) was uniformly labeled on the benzene ring. The radioactive compounds and their unlabeled derivatives were furnished by CIBA-Geigy, Ltd., Basle, Switzerland. Before use each radiolabeled material was purified on TLC using the system appropriate to each

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compound, as shown in Figures 1–3.

Unlabeled reference compounds which were judged to be potential metabolites were synthesized as follows: *N*-(3-chloro-4-methylphenyl)-*N'*-formyl-*N'*-methylurea was prepared by boiling under reflux (60–70 °C) equimolar quantities of *N*-methylformamide (Eastman) and 3-chloro-*p*-tolyl isocyanate (Aldrich Chemical Co., Milwaukee, Wis.) in anhydrous diethyl ether with constant stirring for 2 h. The desired product was separated by TLC on silica gel by a solvent system of diethyl ether–petroleum ether–acetic acid (20:5:1), R_f 0.55. Extraction of the compound from the silica gel with ethyl acetate followed by recrystallization from ethanol afforded the pure compound with mp 100–101 °C: infrared spectrum (in KBr) peaks at 1720 and 1684 cm^{-1} (both for –C=O); NMR spectrum (in $CDCl_3$) δ 3.23 (NCH_3), 8.50 (NCHO); mass spectrum *m/e* 226 (molecular ion), 167 ($CH_3ClPhNCO$); formaldehyde test (Krueger, 1949), positive. Anal. Calcd for $C_{10}H_{11}N_2O_2Cl$: C, 53.08; H, 4.91; N, 12.39. Found: C, 52.53; H, 5.00; N, 12.17.

N-(3-Chloro-4-methylphenyl)-*N'*-formylurea was prepared by adding dropwise an equimolar amount of 3-chloro-*p*-tolyl isocyanate to formamide (Eastman) in anhydrous diethyl ether with constant stirring at room temperature for 2 h. The desired product was separated by TLC on silica gel in a solvent system of diethyl ether–petroleum ether–acetic acid (20:5:1), R_f 0.47. Extraction with ethyl acetate and recrystallization from absolute ethanol gave off-white crystals with mp 165–167 °C: ir (in KBr) peaks at 1720 and 1695 cm^{-1} (both for –C=O); mass spectrum *m/e* 212 (parent), 167 ($CH_3ClPhNCO$); formaldehyde test, positive. Anal. Calcd for $C_9H_9N_2O_2Cl$: C, 50.82; H, 5.2. Found: C, 51.70; H, 4.6.

N-(3-Trifluoromethylphenyl)-*N'*-formyl-*N'*-methylurea was prepared by adding dropwise an equimolar amount of trifluoro-*m*-tolyl isocyanate (Aldrich Chemical Co., Milwaukee, Wis.) to *N*-methylformamide in anhydrous diethyl ether followed by constant stirring for 1 h at 25 °C. The desired product was separated by TLC on silica gel in a solvent system of methylene chloride–acetone (4:1), R_f 0.85. Extraction with ethyl acetate and recrystallization from absolute ethanol gave the pure compound with mp 154–155 °C: ir (in KBr) peaks at 1725 and 1675 cm^{-1} (for –C=O); mass spectrum *m/e* 246 (molecular ion), 187 (CF_3PhNCO), 161 (CF_3PhNH_2); NMR (in $CDCl_3$) δ 3.23 (NCH_3), 8.1 (NCHO).

N-(3-Trifluoromethylphenyl)-*N'*-formylurea was prepared by adding dropwise an equimolar amount of trifluoro-*m*-tolyl isocyanate to formamide in anhydrous diethyl ether followed by constant stirring for 2 h at 25 °C. The desired product was obtained after separation by TLC on silica gel in methylene chloride–acetone (4:1), R_f 0.68, extraction with ethyl acetate, and recrystallization from ethanol with mp 178–180 °C: ir (in KBr) peaks at 1700 and 1625 cm^{-1} (–C=O); mass spectrum *m/e* 232 (molecular ion), 187 (CF_3PhNCO), 161 (CF_3PhNH_2).

Cell Cultures. Human embryonic lung (HEL) cells were supplied by Flow Laboratories, Rockville, Md. These cells were freshly explanted from in vivo sources and seeded in tissue culture flasks with nutrient medium (North and Menzer, 1972). Protein concentrations were determined by the method of Lowry et al. (1951) using bovine serum albumin (Nutritional Biochemicals) as the standard reference for quantitation.

Incubation Conditions. Radioactive chlorotoluron, fluometuron, or metobromuron, respectively (100 000–300 000 dpm), was added to culture flasks by dispersing the material carefully over one surface of a flask upon which the cells would be seeded. The solvent was then evaporated under sterile conditions. Two-week old HEL cells were removed by trypsinization from a donor flask and together with 2 ml of nutrient medium were added to culture flasks which were previously inoculated with radioactive compounds. The flasks were tightly stoppered and the cultures were maintained in an incubator at 37 °C for periods of 6 to 72 h. Control flasks containing all the above components except cells were similarly prepared and incubated.

Metabolism of chlorotoluron, fluometuron, and metobromuron was also investigated using HEL cell sonicates. Primary HEL cells were allowed to grow to maximum density and then were scraped into suspension in the nutrient medium. Culture medium and cells were pooled and centrifuged at 280*g* at 5 °C for 10 min. The supernatant was decanted and the cells were resuspended in a 0.05 M Tris-HCl (pH 7.4) buffer with subsequent recentrifugation. This washing process was repeated 4 times in order to remove serum protein from the cells. The cells were then resuspended in 15 ml of Tris-HCl buffer and transferred to a Branson rosette container in which the cells were disrupted by sonic vibrations from the tip of a Branson sonifier (Branson Sonic Power, Danbury, Conn.) (North and Menzer, 1970). The sonicate was decanted into a 25-ml Erlenmeyer flask which contained 200 000 dpm of [^{14}C]chlorotoluron, [^{14}C]fluometuron, or [^{14}C]metobromuron, 36 μmol of NADPH, and 3 μmol of $MgCl_2$ (North and Menzer, 1972). The final volume in the flask was 3 ml. The flask and contents were incubated at 37 °C with shaking for 3 h. The controls contained the same components except that the cell sonicates had been boiled. The metabolites were extracted into ether and radioactivities in the ether and aqueous solutions were assayed in a liquid scintillation spectrometer.

Conjugative metabolism of chlorotoluron, fluometuron, and metobromuron was also investigated using HEL cell sonicates. The cell sonicates were prepared as described before. Two-milliliter cell sonicates in 0.05 M Tris-HCl buffer (pH 7.4) were poured into a 50-ml Erlenmeyer flask which contained 200 000–300 000 dpm of [^{14}C]chlorotoluron, [^{14}C]fluometuron, or [^{14}C]metobromuron, 36 μmol of NADPH, and 3 μmol of $MgCl_2$. The final volume in each flask was 5 ml. The flask contents were incubated at 37 °C in a water bath with constant shaking for 3 h. At the end of the incubation, 36 mmol of UDPGA solution (in 0.05 M, pH 7.4, Tris-HCl buffer) was added to each flask except for the control flasks. The final pH stayed around 7.4 to 7.5. The flask contents were further incubated at 37 °C with constant shaking for 20 min (Dutton, 1966). The solutions were extracted with anhydrous ether after incubation.

Extraction Procedures. At the end of the preselected periods of incubation, the culture medium was neutralized to pH 7.0 with dilute HCl, and diethyl ether was added to each flask to stop all metabolic activity. The ether layer was separated and the remaining aqueous solution was extracted 4 times with additional equal volumes of ether. Finally, the ether extracts from each single flask were combined into one fraction for each flask. The remaining aqueous solution was adjusted to pH 2 with 0.1 N HCl and further extracted 4 times with equal volumes of diethyl ether to yield another ether extract and the final aqueous phase. The latter aqueous solution was adjusted to pH 4.4 with 0.1 N HCl followed by incubation with β-glucuronidase (1750 units/ml, Nutritional Biochemicals) at 37 °C for 24 h in order to free possible metabolites from conjugation with glucuronic acid. The solution was ex-

Table I. [^{14}C]Chlorotoluron Metabolism by HEL Cells: Percent of Each Ether-Extractable Metabolite Present in Recovered Radioactivity at Corresponding Period of Incubation

Metabolites $CH_3ClPhNHCONR_1R_2$		6 h		12 h		24 h		48 h		72 h	
R_1	R_2	Test	Control	Test	Control	Test	Control	Test	Control	Test	Control
CH_3	CH_3	97.0	98.9	96.0	98.1	94.7	98.6	96.0	97.4	96.5	97.0
CH_3	CHO	0.4	0.1	0.6	0.2	1.0	0.2	0.6	0.2	0.4	0.3
CH_3	H	0.5	0.1	0.9	0.4	1.0	0.3	0.7	0.4	0.6	0.5
CHO	H	0.6	0.0	0.8	0.1	0.7	0.1	0.7	0.2	0.6	0.3
H	H	0.1	0.0	0.2	0.1	0.5	0.1	0.3	0.2	0.3	0.3
Unknown		0.0	0.0	0.2	0.1	0.3	0.1	0.2	0.1	0.2	0.1
Unknown (origin)		0.7	0.7	0.5	0.6	0.5	0.4	0.5	0.5	0.7	0.7
Total organo-soluble		99.3	99.8	99.2	99.6	98.8	99.8	99.0	99.0	99.3	99.2
Total water soluble		0.7	0.2	0.8	0.4	1.2	0.2	1.0	1.0	0.7	0.8

tracted with ether as before. Radioactivity in the ether extracts and the aqueous solution was assayed in a liquid scintillation spectrometer. In cases of isolation and purification of metabolites for mass spectral analysis, ethyl acetate was used as the solvent to extract metabolites.

Chromatography. Commercially prepared TLC plates (silica gel F-254, 0.25 mm thickness, E. Merck) were used to resolve ether-extractable metabolites. No attempt was made to separate water-soluble metabolites because of low levels of radioactivity. Two-dimensional chromatography was used for cochromatography in the identification of unknown metabolites. Metabolites were located on TLC plates by radioautography using Kodak No-Screen x-ray film. The location of the unlabeled reference compounds on the TLC plates was visualized by fluorescence under uv light. Solvent systems used to develop TLC plates were: (1) methylene chloride–acetone (4:1); (2) methylene chloride–ethanol (4:1); and (3) chloroform–methanol (9:1).

Radioassay Procedures. A Packard Tri-Carb liquid scintillation spectrometer (Model 3375) was used for assaying radioactivity. Radioactive materials which were soluble in organic solvents were dissolved in a scintillation mixture containing 5 g of PPO (2,5-diphenyloxazole) and 0.3 g of dimethyl POPOP [1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene] per liter of toluene. Radioactivity in the aqueous solution was assayed by dissolving the solution in a mixture of 5.5 g of PPO and 200 mg of dimethyl POPOP per liter of a 2:1 mixture of toluene and Triton X-100 (Packard Instrument Co., Downers Grove, Ill.) Radioassay of metabolites on silica gel was accomplished by scraping radioactive areas from TLC plates and pulverizing and dispersing the silica gel in scintillation counting solution with a 4.0% thixotropic gel (Cab-O-Sil, Packard Instrument Co.). External standardization was used to correct for quenching.

RESULTS

Recovery of Administered Radioactivity. After various periods of incubations with HEL cells of chlorotoluron, fluometuron, and metobromuron, respectively, all flask contents were extracted with anhydrous diethyl ether. Good recovery of administered radioactivity was achieved with each radioactive compound after every incubation period. Over 99% of the originally inoculated radioactivity was regained from both the controls and the test flasks. The ether fractions contained over 98% of the recovered radioactivity while the aqueous fractions contained less than 2%. Despite low percentages, radioactivities from the aqueous fractions of test flasks generally increased with incubation for the first 24 h (Tables I, II, and III). Recovery of inoculated radioactive compounds from control flasks averaged over 99%, of which 99.6% was in the ether fraction and 0.4% in the aqueous phase.

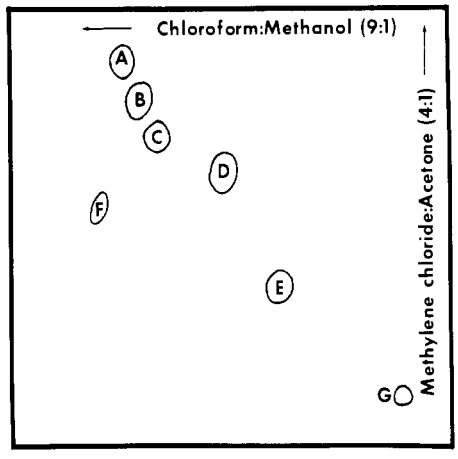

Figure 1. Two-dimensional thin-layer chromatogram of chlorotoluron and its derivatives. Explanation: (A) *N*-(3-chloro-4-methylphenyl)-*N'*-formyl-*N'*-methylurea; (B) chlorotoluron; (C) *N*-(3-chloro-4-methylphenyl)-*N'*-formylurea; (D) *N*-(3-chloro-4-methylphenyl)-*N'*-methylurea; (E) *N*-(3-chloro-4-methylphenyl)urea; (F) unknown; (G) origin.

To free metabolites from possible conjugations, aqueous fractions were treated with β-glucuronidase followed by extractions with diethyl ether. The total recovery of radioactivity was high but the radioactivity in the ether fraction was too low for identification of any metabolites.

To scrutinize possible glucuronic acid conjugation of the phenylurea metabolites, UDPGA was added to HEL cell sonicates which had been fortified with NADPH, [^{14}C]-chlorotoluron, [^{14}C]fluometuron, or [^{14}C]metobromuron and incubated as described. The mixtures were then extracted with ether, and both ether and aqueous fractions were radioassayed. The result indicated that the final aqueous solution from test flasks contained no more radioactivity than that found in the control flasks containing no cofactor, UDPGA.

Chemical Nature of Metabolites. Two-dimensional thin-layer cochromatography and chemical characterization procedures were used to elucidate and identify the nature of the phenylureas and their metabolites.

Chlorotoluron Metabolites. Thin-layer chromatography resolved the ether extractable radioactivity into seven spots (Figure 1, Table I). Cochromatography indicated that one of the spots was the parent compound, chlorotoluron, which contained the majority of the radioactivity (over 99%) for all incubation periods. Isolation, purification, and mass spectral analysis also proved this spot to be chlorotoluron. Four of the other spots were isolated from thin-layer plates. Cochromatography suggested that these four spots were identical with *N*-(3-chloro-4-methyl-

Table II. [^{14}C]Fluometuron Metabolism by HEL Cells: Percent of Each Ether-Extractable Metabolite Present in Recovered Radioactivity at Corresponding Period of Incubation

Metabolites $CF_3PhNHCONR_1R_2$		6 h		12 h		24 h		48 h		72 h	
R_1	R_2	Test	Control	Test	Control	Test	Control	Test	Control	Test	Control
CH_3	CH_3	96.2	97.1	95.3	96.6	95.0	96.7	95.7	96.3	95.3	95.8
CH_3	CHO	0.6	0.6	0.7	0.6	0.7	0.6	0.6	0.5	0.6	0.4
CH_3	H	2.2	2.0	2.4	2.0	2.6	2.1	2.6	2.3	2.7	2.6
H	H	0.2	0.0	0.2	0.0	0.2	0.0	0.2	0.1	0.3	0.2
Unknown (origin)		0.0	0.0	0.0	0.0	0.2	0.1	0.1	0.1	0.1	0.1
Total organo-soluble		99.2	99.7	98.6	99.2	98.7	99.5	99.2	99.3	99.0	99.1
Total water soluble		0.8	0.3	1.4	0.8	1.3	0.5	0.8	0.7	1.0	0.9

Table III. [^{14}C]Metobromuron Metabolism by HEL Cells: Percent of Each Ether-Extractable Metabolite Present in Recovered Radioactivity at Corresponding Period of Incubation

Metabolites $BrPhNHCONR_1R_2$		6 h		12 h		24 h		48 h		72 h	
R_1	R_2	Test	Control	Test	Control	Test	Control	Test	Control	Test	Control
OCH_3	CH_3	96.8	97.3	95.3	97.5	95.3	97.8	95.7	97.6	96.0	97.4
OH	CH_3	0.6	0.4	0.5	0.3	0.5	0.2	0.2	0.2	0.3	0.3
H	CH_3	1.3	1.1	1.8	1.1	1.4	1.1	1.3	1.1	1.2	1.0
H	H	0.5	0.3	0.8	0.3	0.7	0.3	0.9	0.5	0.9	0.6
Unknown (origin)		0.2	0.2	0.2	0.1	0.3	0.3	0.2	0.2	0.2	0.1
Total organo-soluble		99.4	99.3	98.6	99.3	98.2	99.7	98.3	99.6	98.6	99.4
Total water soluble		0.6	0.7	1.4	0.7	1.8	0.3	1.7	0.4	1.4	0.6

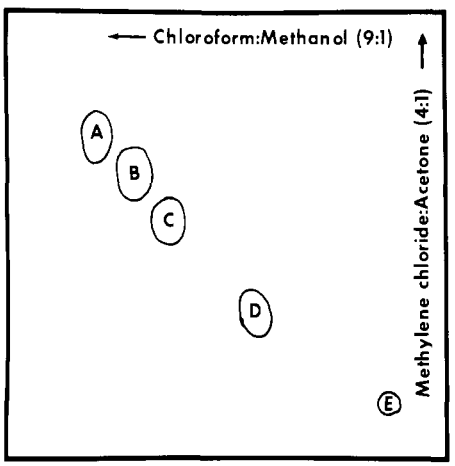

Figure 2. Two-dimensional thin-layer chromatogram of fluometuron and its derivatives. Explanation: (A) *N*-(3-trifluoromethylphenyl)-*N'*-formyl-*N'*-methylurea; (B) fluometuron; (C) *N*-(3-trifluoromethylphenyl)-*N'*-methylurea; (D) *N*-(3-trifluoromethylphenyl)urea; (E) unknown (origin).

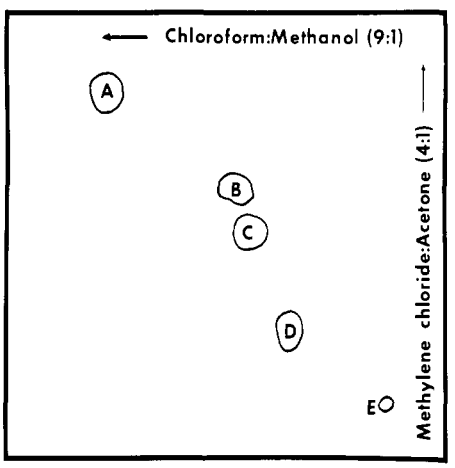

Figure 3. Two-dimensional thin-layer chromatogram of metobromuron and its derivatives. Explanation: (A) metobromuron; (B) *N*-(4-bromophenyl)-*N'*-hydroxy-*N'*-methylurea; (C) *N*-(4-bromophenyl)-*N'*-methylurea; (D) *N*-(4-bromophenyl)urea; (E) unknown (origin).

phenyl)-*N'*-formyl-*N'*-methylurea, *N*-(3-chloro-4-methylphenyl)-*N'*-formylurea, *N*-(3-chloro-4-methylphenyl)-*N'*-methylurea, and *N*-(3-chloro-4-methylphenyl)urea. The structure of the metabolite, *N*-(3-chloro-4-methylphenyl)-*N'*-methylurea, was also proved by mass spectral analysis. The two metabolites corresponding to the two formyl derivatives on TLC were treated with 0.1 N HCl (to pH 4.0) and heated at 70 °C for 2 h. After isolation with ether and separation of the products on TLC, it was found that HCl treatment had transformed the *N'*-formyl-*N'*-methylurea and *N'*-formylurea derivatives into *N'*-methylurea and urea derivatives, respectively. Two other spots, including the one at the origin, were not identified. The majority of the metabolites appeared within 24 h after incubation.

Fluometuron Metabolites. Thin-layer chromatography revealed four ether extractable metabolites (Figure 2, Table II). Cochromatography identified three of the metabolites as *N*-(3-trifluoromethylphenyl)-*N'*-formyl-*N'*-methylurea, *N*-(3-trifluoromethylphenyl)-*N'*-methylurea, and *N*-(3-trifluoromethylphenyl)urea. Chemical characterization using 0.1 N HCl solution and heating at 70 °C converted *N*-(3-trifluoromethylphenyl)-*N'*-formyl-*N'*-methylurea to *N*-(3-trifluoromethylphenyl)-*N'*-methylurea. Reduced concentrations of the parent compound remaining after each incubation period indicated that the metabolism rate was most rapid in the first 24-h incubation period.

Metobromuron Metabolites. There were four metabolites as detected by thin-layer chromatography (Figure 3, Table III). Three of the metabolites were identified cochromatographically as *N*-(4-bromophenyl)urea, *N*-(4-bromophenyl)-*N'*-methylurea, and *N*-(4-bromophenyl)-*N'*-hydroxy-*N'*-methylurea. When the metabolite identical with *N*-(4-bromophenyl)-*N'*-hydroxy-*N'*-methylurea was allowed to react at 37 °C with dimethyl sulfate in sodium

Table IV. Protein Contents per Flask of HEL Cell Cultures which Were Incubated with a Single Dose of Chlorotoluron (7.6 μg), Fluometuron (5 μg), or Metobromuron (9 μg)

Compounds	Protein content (μg) after incubation for h					
	0	6	12	24	48	72
Chlorotoluron	20	25	55	100	150	200
Fluometuron	20	40	55	80	100	200
Metobromuron	20	35	100	142	160	160
Control	20	46	66	89	150	200

hydroxide, *N*-(4-bromophenyl)-*N'*-methoxy-*N'*-methylurea was formed (70–80% yield). Metabolism also occurred more rapidly in the first 24-h incubation period.

Protein Contents in HEL Cell Cultures. Protein contents continued to increase through 72 h in the HEL cells which were treated with a single dose of chlorotoluron (7.6 μg), fluometuron (5 μg), or metobromuron (9 μg) (Table IV). This indicated that cell growth was not inhibited by these three phenylurea compounds at the concentrations used.

DISCUSSION

Chlorotoluron, fluometuron, and metobromuron were very resistant to metabolism by HEL cells, since less than 2% of the applied compound was metabolized in 72 h after incubation. The greatest concentrations of metabolites were formed between 12 and 24 h after incubation. It seemed that only oxidative dealkylation of carbamoyl group substituents was involved in the metabolism of these three phenylurea molecules. Chlorotoluron has also been shown to be resistant to metabolic attack in rat hepatic microsomal systems (Hinderer and Menzer, 1976a), although it is readily degraded in rats in vivo (Mücke et al., 1976).

Conjugation of any type of these compounds by HEL cells was insignificant. The addition of the glucuronic acid donor, UDPGA, to the cell preparations (sonicates) did not afford any increase in radioactivity in the aqueous fraction. Furthermore, β-glucuronidase did not free any metabolites from conjugation. These facts make extremely unlikely the formation of glucuronide conjugates of these phenylurea compounds in the HEL cell cultures.

Changing patterns of metabolite concentrations in the cases of chlorotoluron and fluometuron seemed to reveal that *N*-formyl derivatives were intermediate species resulting from direct oxidation of *N*-alkyl groups. Dealkylation followed the oxidation and formed the dealkyl derivatives. *N*-Hydroxymethyl derivatives were not found; if formed, they were apparently too unstable to be detected.

Metobromuron metabolism did not result in *N*-formyl derivatives. The reason may be that the molecule contains an *N*-methoxyl group ($NOCH_3$) as well as an *N*-methyl group. Demethoxylation and demethylation were the only mechanisms responsible for identified metabolites of metobromuron by HEL cells.

Although spontaneous degradation of the phenylurea compounds in the controls seemed to be high, the fact that the concentrations of metabolites being formed by HEL cell cultures were consistently greater than those from controls demonstrated that metabolism was indeed due to the action of the HEL cells. Oxidative metabolism seemed to predominate at least in that no metabolites that were products of other possible metabolic events were identified. Of course the radioactivity in the aqueous fraction was unidentified and this material may result from other than oxidative mechanisms.

The behavior of these compounds in HEL cells may be compared with the results of similar studies with dimethoate, carbaryl, and chlordimeform (North and Menzer, 1972; Lin et al., 1975a,b), in which oxidative reactions predominated, to the virtual exclusion of other metabolic events. An important difference was noted, however, in the much higher activity of the cells in metabolizing these other pesticides relative to the phenylureas in the present study.

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