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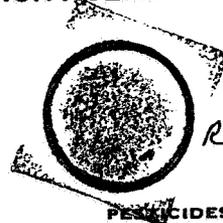
7607/Linuron SS

CASWELL FILE
6/25/85

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

004506

JUN 25 1985



Releasable

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Expedited Review of Linuron Rat Metabolism Study
Accession # 257451; Caswell # 528

TO: Ingrid Sunzenauer, Review Manager #78
Special Review Branch (TS-767C)
and
Robert Taylor, PM #25
Registration Division (TS-767C)

FROM: James N. Rowe, Ph.D. *James N. Rowe 6/24/85*
Section V, Toxicology Branch/HED (TS-769C)

DJ 6/24/85

THRU: Laurence D. Chitlik, D.A.B.T.
Section Head, Section V
Toxicology Branch/HED (TS-769C)
and

fpc 6/25/85

M. 6/25/85

Theodore M. Farber, Ph.D.
Chief, Toxicology Branch/HED (TS-769C)

ACTION REQUESTED: Expedite request of 6/10/85 for review of linuron rat metabolism study.

RECOMMENDATIONS: This study is classified as Core-Supplementary data. Although some useful information is obtained relative to the metabolism of linuron, numerous deficiencies in the conduct of this study were noted, and a repeat study is required. Specifically, the following major deficiencies were noted:

- 1) Only 2 rats/sex/group were studied, in contrast to current Guidelines which recommend 5 rats/sex/group.
- 2) A large fraction (i.e. about 60%) of the excreted label was unidentified as to structure. In view of current analytical technologies, this percentage seems unreasonable.
- 3) The repeated dose group (Group C of 1982 Guidelines) received the cold and labelled test substance at two different dose levels and by two different routes of administration, which complicates interpretation of the data.
- 4) No individual animal data were submitted.

Numerous additional deficiencies are detailed in the "Methods" section of the appended review.

Excerpts of data submitted by *dufont* on *Linuron* were included in this review. (*11* pages). These pages may be requested by writing Freedom of Information (A-101), EPA, Washington, D.C. 20460. Requesters will be asked to sign an Affirmation of Non-multinational Status.
See D-7608B for excerpts

Discussion

Although the investigators were able to characterize some of the excreted metabolites, the metabolic pathway proposed in the submitted study is premature in the opinion of this reviewer in view of the large percentage of excreted radiolabel that was unidentified.

A concern was previously raised regarding possible contamination of technical linuron by dichloroaniline (DCA), hydroxy-dichloroaniline (OH-DCA), tetrachloroazobenzene (TCAB) and tetrachloroazoxybenzene (TCAOB). These compounds have been associated with numerous toxic effects. Although none of these compounds were identified in the submitted study, because of the deficiencies stated above, this conclusion should be considered tentative.

Linuron appears to pose little hazard for bioaccumulation, as excretion is relatively rapid (half-life of excretion = 21-56 hours), and residues in tissues were minimal. No accumulation in testes, a potential target organ, was apparent.

DATA EVALUATION RECORD

004506

STUDY TYPE: Rat metabolism.

CHEMICAL: (Phenyl-¹⁴C[U]) linuron; 1-[3,4-dichlorophenyl]-3-methoxy-3-methylurea; Caswell # 528

TEST MATERIAL: Uniformly labeled (phenyl ring) linuron with a specific activity of 6.1 uCi/mg and a stated purity of >99% (Agricultural Department Radiochemical Inventory File #146); unlabeled linuron with a stated purity of >98%; synthesized at the duPont Experimental Station.

STUDY IDENTIFICATION:

Animal species: male and female Charles River CD rats from Charles River Breeding Laboratories (Kingston, N.Y.); 2/sex/dose.

Laboratory: 1) Dosing and sample collection: Haskell Laboratory
2) Isolation, identification and quantitation: Agricultural Chemicals Department, Research Division, Experimental Station, Wilmington, Delaware 19898

Study No.: AMR 250-84

Date of report: 3/27/85

Study Director: L.G. Carter

EPA #s: Caswell #528; Accession # 257451

CONCLUSIONS:

¹⁴C-Linuron(UL), when administered orally (gavage) to male and female rats as a single dose of 24 mg/kg or 400 mg/kg, or as a single dose of 24 mg/kg after 21 days of preconditioning with 100 ppm unlabeled compound in the diet, was rapidly absorbed, metabolized and excreted. The primary route of excretion was the urine (60-76%), and the feces (13-25%) was the secondary route of elimination. No ¹⁴CO₂ was detected. The degree of gastrointestinal absorption appears to be significant since the ratio of radioactivity found in the urine to that in the feces ranges from 2.4 to 5.

Biological half-lives of excretion were different between low dose (24 mg/kg) males and females, 21 and 35 hours, respectively. The high dose group (400 mg/kg) had a half-life of approximately 55 hours with no apparent difference between sexes.

A large percentage (up to 60%) of excreted metabolites, urinary and fecal, were not identified. Therefore, in the opinion of this reviewer, speculation as to the metabolic pathway of linuron is premature. The major fecal metabolites that were identified were NOR-linuron (1-[3,4-dichlorophenyl]urea; up to 38% of label/fecal sample) and OH-NOR-linuron (4,5-dichloro-2-hydroxyphenylurea; up to 26% of label/fecal sample). OH-NOR linuron was the major urinary metabolite (up to 53% of label/urine sample). Structures of metabolites and other compounds of interest are appended as Figure 1 (photocopied from the study report).

Less than 1% of the administered dose remained in the body at either 96 or 120 hours post-dosing, suggesting that linuron poses little hazard for bioaccumulation.

BACKGROUND

Data concerning the formation of three major contaminants in linuron of 3,4-dichloroaniline (DCA), 3,3',4,4'-tetrachloroazobenzene (TCAB) and 3,3',4,4'-tetrachloroazoxybenzene (TCAOB) has led the Agency to evaluate their hazards. DCA is one of 20 substituted anilines considered by the EPA Interagency Testing Committee (ITC) for oncogenicity, mutagenicity, teratogenicity, reproductive effects and chronic effects emphasizing blood effects, e.g., acute methemoglobinemia, which is characteristic of anilines. TCAB AND TCAOB may have reproductive, teratogenic, and carcinogenic potential, and have been implicated in several industrial incidents in which chloroacne has been reported. The registrant is aware of our concerns over these contaminants and therefore has explicitly addressed them in this study.

EXPERIMENTAL PROCEDURES/COMMENTS:

A copy of the experimental procedures are attached. The submitted methods were reviewed, and the following comments are made:

1. Only 2 animals/sex/group are tested as opposed to 10 (5 of each sex) recommended by the 1982 Pesticide Assessment Guidelines.
2. An intravenous dose group ("Group A" of the 1982 Guidelines) was not studied because the test substance was not soluble in saline or water, and therefore this group can be omitted.
3. The repeated, unlabeled low dose (Group II) should be the same as the labeled dose, i.e. 24 mg/kg. In the present study, a diet containing 100 ppm (approximately 5 mg/kg) was given rats, followed by the labeled dose. Further, the preconditioning doses should be administered by gavage, and not in the diet which could produce a different rate of absorption and/or metabolism.
4. It is not possible to fully assess this study since individual animal data were not provided.
5. Rats were sacrificed by chloroform exposure. Asphyxiation by CO₂ is the more common method.
6. The 96 and 120 hour sacrifice times were established on the basis of a pilot study which was not submitted with the study.
7. A full description of the sensitivity/precision of all procedures should be submitted. For example, in one of the representative TLC chromatograms there is an overlap of metabolite areas. How are these metabolites separated?
8. It was stated that selected samples were taken (in feces). How were these selected to be representative and how many were taken?
9. Identification of the polar origin and unidentified metabolites (UNI-III) is critical to understanding the metabolic pathways and to clearly establishing that metabolites/contaminants of particular concern, i.e., DCA, OH-DCA, TCAB, TCAOB. Therefore, it would seem necessary to do additional work to identify more fully the unknown metabolites mentioned above.

RESULTS

Radioactivity recoveries: Radioactive recoveries (excreta, tissue residues, and cage rinses) in Groups I and II at 96 hours post-dosing averaged from 85-96%, respectively, with no apparent difference between males and females (see Table 1 of this review). The authors reported a 70% recovery in one Group I female; no explanation for this apparent low recovery was provided. Since the average recovery for this group was 85%, and only 2 females were studied, recovery from the other female of this group must have been near 100%. Since individual animal data were not provided, this finding cannot be further evaluated. Recovery in Group III rats was 91% in males and 100% in females at 120 hours after treatment (Table 1). Group III rats were sacrificed at 120 hours rather than 96 (as for Groups I and II) apparently because a pilot study (data not submitted) indicated that a longer study period was necessary to recover the maximum amount of radiolabel.

In all but one animal, where the skin was reportedly contaminated with excrement, greater than 90% of the recovered radioactivity in all treatment groups was found in the excreta (urine=60-76%, feces=13-25%). This finding suggests that a significant amount of the administered linuron is absorbed systemically. No radioactivity was detected as $^{14}\text{CO}_2$, indicating that respiration was not a major metabolic route.

Table 1. Recovery of Radiolabel^a

<u>Excretion</u>	<u>Group I^b</u>		<u>Group II^b</u>		<u>Group III^c</u>	
	<u>Male</u>	<u>Female</u>	<u>Male</u>	<u>Female</u>	<u>Male</u>	<u>Female</u>
Urine 6 hrs	3.55	1.82	3.70	0.37	0.48	0.19
24	40.43	21.49	42.38	20.91	7.15	6.58
48	13.97	38.05	21.15	45.63	28.28	25.30
72	1.67	3.88	2.85	5.44	23.43	35.74
96	0.71	1.70	1.37	1.90	5.79	6.54
120	-	-	-	-	4.24	1.42
Urine total	60.33	66.94	71.46	74.25	69.37	75.77
Feces 6 hrs	0.26	0.01	0.00	0.20	0.01	0.02
24	17.26	2.80	8.82	7.04	2.98	1.26
48	7.05	9.08	6.10	6.98	5.44	0.88
72	0.41	1.18	2.20	1.31	7.58	10.48
96	0.96	0.26	0.41	0.39	1.18	3.06
120	-	-	-	-	0.30	0.93
Feces total	25.17	13.32	17.53	15.92	17.50	16.62
Tissues	0.76	1.43	5.78	0.90	1.83	2.35
Cage washings	1.58	3.41	1.49	1.87	2.27	5.12
Total Recovery	87.84	85.10	96.26	92.94	90.97	99.86

^adata excerpted from submitted study. Values are % of administered dose.

^banimals sacrificed at 96 hours.

^canimals sacrificed at 120 hours.

Radioactivity retained in tissues/organs

Average radioactivity retained in the tissues and organs of the 3 treatment groups ranged from roughly 1-6% of the administered dose (Table 2 of this review). The authors stated that if the one Group II male rat with 10.1% retention (due to contamination of the hide with excreta) is omitted, <2% of the applied radioactivity was retained in Groups I and II. The highest reported retention in Group III was 2.8%. No individual animal data were submitted, therefore these observations can not be verified independently by the reviewer.

The concentration of radiolabel in heart, lungs, kidney, and liver in Group I and II males averaged from 0.15 to 0.51 ppm, with no apparent difference between these two groups. The concentration of label in these organs from Group I and II females averaged from 0.23 to 1.33 ppm. From 0.2 to 0.3% of the administered dose was unaccounted for in the carcass. The hide of Group II males contained about 3.9 ppm (5.1% administered dose), reportedly due to contamination by excreta.

The testes, which were a target tissue for oncogenic effects in the 2-year chronic feeding study (benign testicular adenomas) retained 0.29 and 0.05 ppm in Group I and II males, respectively. This apparent decrease in concentration of label after preconditioning, provided it is not due to the small number of animals utilized, suggests a possible saturation of a compartment in the testes, since other organs retained similar concentrations of label with or without preconditioning. Blood, which is an apparent target for linuron toxicity in the form of sulf- and methemoglobinemia, retained from 0.20 to 0.45 ppm of radiolabel in Group I and II rats.

High dose rats (Group III) retained a greater concentration of label in their tissue as compared to Groups I or II, however similar amounts of label were retained in all 3 groups if expressed as % dose. The highest concentration of label was noted in hide, 20.0 and 38.3 ppm in males and females, respectively. Label retained by heart, lungs, kidneys and liver averaged from 1.87 to 6.13 ppm in males and 2.73 to 8.93 in females. Testes from these rats retained 1.00 ppm, whereas blood contained 3.64 and 3.95 ppm in males and females, respectively.

Table 2. Retention of ^{14}C -Radiolabel by Tissues and Organs^a

Tissue	Group I		Group II		Group III	
	Male	Female	Male	Female	Male	Female
Brain	0.02 ^b (<0.01) ^c	0.05 (<0.01)	0.03 (<0.01)	0.06 (<0.01)	0.60 (<0.01)	0.20 (<0.01)
Fat	0.01 (<0.01)	0.01 (<0.01)	0.07 (<0.01)	0.12 (<0.01)	0.87 (<0.01)	1.00 (<0.01)
GI tract	0.36 (0.17)	1.10 (0.45)	0.28 (0.19)	0.32 (0.14)	4.67 (0.18)	3.73 (0.11)
Heart	0.16 (<0.01)	0.29 (<0.01)	0.15 (<0.01)	0.23 (<0.01)	1.87 (<0.01)	2.73 (<0.01)
Kidneys	0.51 (0.01)	1.22 (0.03)	0.37 (0.02)	0.37 (0.02)	6.13 (0.02)	7.00 (0.02)
Liver	0.44 (0.09)	1.33 (0.22)	0.51 (0.14)	1.00 (0.15)	6.13 (0.10)	8.93 (0.11)
Lungs	0.27 (0.01)	0.50 (0.01)	0.26 (0.01)	0.46 (0.01)	4.53 (0.01)	4.07 (0.01)
Muscle	0.10 (0.01)	0.13 (0.01)	0.04 (<0.01)	0.06 (<0.01)	0.53 (<0.01)	0.67 (<0.01)
Spleen	0.19 (<0.01)	0.55 (<0.01)	0.22 (<0.01)	0.35 (<0.01)	4.47 (<0.01)	4.60 (<0.01)
Gonads	0.29 (<0.01)	0.33 (<0.01)	0.05 (<0.01)	0.25 (<0.01)	1.00 (<0.01)	3.07 (<0.01)
Bone	0.06 (<0.01)	0.10 (<0.01)	0.05 (<0.01)	0.07 (<0.01)	0.73 (<0.01)	0.93 (<0.01)
Blood	0.30 (0.05)	0.37 (0.05)	0.20 (0.04)	0.45 (0.07)	3.64 (0.04)	3.95 (0.03)
Hide	0.19 (0.20)	0.23 (0.43)	3.94 (5.06)	0.37 (0.28)	20.00 (1.17)	38.26 (1.89)
Carcass	0.07 (0.22)	0.11 (0.23)	0.10 (0.32)	0.11 (0.23)	1.87 (0.31)	1.20 (0.18)

^adata excerpted from tables IV-VI of submitted study.

^bppm of label (ug linuron equivalent/g tissue).

^cpercent of administered dose.

Rate of excretion

Estimates of the biological half life ($t_{1/2}$) reported are presented below:

	<u>Sample</u>	<u>$t_{1/2}$(hours)</u>
Grp I	Male	21
	Female	36
Grp II	Male	22
	Female	34
Grp III	Male	54
	Female	56

These values appear correct based on inspection of figures 5-10 from the study report, which compare the excretion of label vs. time in the dose groups. Male rats excrete linuron more rapidly than females at the low dose (24 mg/kg). At the high dose (400 mg/kg) no difference between male and female excretion rates is evident. This longer half-life in high dose rats may be due to a saturation of the metabolic pathways or effects on absorption from the GI tract.

Metabolite Identification- Fecal Samples

A substantial percentage of radioactivity excreted in the feces was unidentified. The origin contained up to 35%, 33% and 48% of the applied label in Group I, II, and III rats respectively. That is, this amount of radioactivity did not move with the solvent front on the thin-layer chromatogram, and may contain several polar metabolites that are unidentified.

Metabolite distributions are summarized in Tables 3a and 3b of this review. Seven distinct metabolites and the parent compound were identified in feces, however as noted above, the origin contained as much as 48% of the fecally excreted label as unidentified metabolite(s). In addition, two unidentified metabolites distinct from the origin ("UNI" and "UNIII") were detected at concentrations as high as 19.0% of the fecally excreted label. Thus, the majority of the label excreted in feces was unidentified.

No difference between males and females, or effect of treatment paradigm on the excretion of label in the origin could be reliably ascertained. Group I and II females appeared to excrete more of the UNIII metabolite at 24 and 48 hours than did males or Group III males and females, however because of the small number of rats studied (2/sex), the significance of these apparent differences is unclear.

For metabolites that were identified in feces, the predominant structures were NOR linuron and OH-NOR linuron. Group I and II females appeared to form more NOR linuron than did males at 24 and 48 hours. Group III animals excreted more label as NOR linuron than groups I or II, however no difference between the sexes was apparent. In contrast, more OH-NOR linuron appeared to be excreted in the feces of Groups I and II males than females or Group III males and females at 24 and possibly 48 hours. Other minor fecal metabolites included OH-desmethoxy linuron, desmethoxy linuron, OH-desmethyl linuron, desmethyl linuron, OH-linuron and the parent compound linuron were reported in small amounts. No evidence of

TCAB, TCAOB, DCA OR OH-DCA was reported in any of these fecal samples. The reference standards were limited to the 2-hydroxyphenyl compounds. This choice of reference standards should be reconsidered in light of the large percentages of unidentified polar and unknown metabolites. No individual animal data were submitted to determine the range of values or verify the metabolite pattern.

Table 3a. Excretion of Metabolites in Feces^a

Metabolite	Group	Hours After Treatment				
		24	48	72	96	120
Origin	I	31.7/25.3 ^b	30.0/23.0	27.8/29.7	35.3/34.6	
	II	30.0/26.1	27.6/29.9	23.4/32.9	27.9/25.9	
	III	20.7/47.6	33.2/40.0	35.6/27.2	30.1/24.2	27.4/24.0
NOR	I	0.0/27.1	1.7/ 8.3	0.0/ 0.0	0.0/ 0.0	
	II	14.0/22.1	4.4/ 8.1	0.0/ 3.0	0.0/ 0.0	
	III	34.5/37.5	15.2/15.8	7.3/ 6.0	3.6/ 2.3	0.0/ 0.0
OH-NOR	I	17.9/ 0.2	25.7/11.4	14.0/10.6	15.2/25.9	
	II	9.3/ 1.6	3.8/11.5	11.0/10.2	18.1/21.2	
	III	0.0/ 6.3	14.4/11.2	17.0/12.3	22.5/ 3.9	36.4/11.1
UNIII	I	5.1/15.2	0.0/15.2	0.0/ 8.8	0.0/ 7.1	
	II	6.5/19.2	11.6/11.8	3.3/ 5.0	0.0/ 0.0	
	III	7.7/ 3.9	3.3/ 4.1	3.8/ 4.8	1.5/ 3.7	0.0/ 3.0
UNI	I	0.8/ 2.8	0.1/ 2.6	0.0/ 2.1	13.1/ 0.0	
	II	3.6/ 4.4	3.2/ 2.2	2.1/ 1.6	0.0/ 0.0	
	III	3.2/ 0.0	0.0/ 4.2	0.0/ 1.9	0.0/ 2.8	4.5/ 3.4

^adata excerpted from submitted study.

^bpercent of fecal radioactivity excreted in males/females.

NOR = 1-(3,4-dichlorophenyl)urea; OH-NOR = 4,5-dichloro-2-hydroxyphenylurea; please refer to Fig. 1 (appended) for structures.

Urinary Metabolites

As noted for fecal excretion, the majority of excreted label was in the form of unidentified metabolites. The origin contained as much as 51% of the extracted radioactivity, with Groups I and II rats appearing to excrete a greater % of extracted label in this form than did group III animals (see Table 3b of this review). Also, a greater relative amount was excreted in this form at 6 hours in all groups as compared to later time points, with the exception of group II females who apparently had no excretion of label in this form at 6 hours. The UNI metabolite composed as much as 15% of the excreted urinary label, with little apparent difference between the sexes, and no apparent effect of the treatment paradigm. The relative % of label excreted in this form appeared to increase for all groups at later time points. Apparently, no UNIII metabolite was detected in the urine of any of the test animals. The UNII metabolite was detected in urine only for Groups I and III (but not II) rats, and generally composed less than 10% of the excreted label. Thus, as was noted

for fecal excretion, a substantial portion of the excreted label was unidentified metabolites.

The major metabolite that was identified in urine appeared to be OH-NOR linuron. Little significant difference between sexes or dose groups was apparent in the relative excretion of this metabolite. An apparent relationship between label excreted as "origin" and as OH-NOR was noted, in that as the relative % excreted as origin decreased the % excreted as OH-NOR increased. Other metabolites excreted in the urine included OH-desmethyl linuron, desmethyl linuron, OH-desmethoxy linuron, desmethoxy linuron, NOR-linuron. No substantial differences between sexes or treatment groups were noted. At 6 hours a possible sex difference in excretion of OH-desmethyl and desmethyl linuron was reported for all test groups, however the effect was not apparent at other time points, and the small number of animals studied precludes any definite conclusion as to these slight differences. The parent linuron was not reported in the urine of any test animals.

Table 3b. Excretion of Metabolites in Urine^a

Metabolite	Group	Hours After Treatment					
		6	24	48	72	96	120
Origin	I	46.4/39.6 ^b	39.0/42.9	21.7/18.6	28.5/22.7	18.0/22.5	
	II	51.3/ 0.0	40.8/38.6	32.7/32.7	24.6/24.8	22.0/23.3	
	III	30.4/33.9	26.4/21.4	21.8/27.6	23.4/16.0	21.7/14.1	17.6/10.1
OH-NOR	I	5.7/21.9	20.9/16.5	39.6/37.9	26.7/39.6	34.5/43.3	
	II	0.0/ 0.0	25.3/19.1	36.8/36.8	47.3/37.3	43.0/35.4	
	III	6.9/11.1	24.3/34.4	34.7/31.0	37.7/39.9	46.0/46.0	52.5/47.2
OH-DES	I	9.7/ 2.5	3.4/ 2.5	7.5/ 1.6	2.8/ 0.4	2.0/ 0.0	
	II	15.8/ 0.0	4.0/ 4.0	0.0/ 0.0	0.0/ 0.0	0.0/ 0.0	
	III	0.0/11.6	2.9/ 2.5	2.5/ 3.8	2.2/ 2.2	0.0/ 0.0	0.0/ 0.0
DES	I	8.1/ 3.4	2.5/ 4.5	2.5/ 2.9	1.8/ 3.1	3.2/ 3.5	
	II	0.9/ 0.0	4.0/ 5.3	3.2/ 3.2	2.7/ 3.6	3.6/ 4.9	
	III	20.1/ 0.0	4.2/ 3.4	3.8/ 3.3	3.9/ 3.5	4.9/ 6.2	2.6/ 7.1
NOR	I	3.6/ 3.8	2.9/ 3.3	2.5/ 4.9	3.7/ 2.2	2.0/ 1.8	
	II	14.0/ 0.0	0.0/ 4.9	1.8/ 2.3	0.0/ 1.8	0.0/ 1.3	
	III	9.4/ 9.9	1.7/ 1.7	1.3/ 1.7	0.9/ 1.7	1.3/ 4.9	0.0/ 4.0
UNI	I	1.6/ 4.6	8.6/ 5.4	7.5/11.1	15.2/12.9	5.9/10.2	
	II	2.2/ 0.0	9.8/ 4.4	9.7/ 9.7	6.4/ 8.2	7.2/ 8.5	
	III	0.0/ 4.5	6.7/ 8.8	7.1/ 4.6	7.4/ 5.2	7.1/ 8.0	7.9/ 7.9
UNII	I	1.2/ 3.8	4.2/ 1.7	1.7/ 0.0	8.3/ 0.0	4.0/ 0.0	
	II	-	-	-	-	-	
	III	0.0/ 4.3	10.1/ 2.5	5.9/ 6.7	4.3/ 6.9	1.8/ 0.0	0.0/ 0.0

^adata excerpted from submitted study.

^bpercent of fecal radioactivity excreted in males/females.

NOR = 1-(3,4-dichlorophenyl)urea; OH-NOR = 4,5-dichloro-2-hydroxyphenylurea; OH-DES = hydroxydesmethyl linuron; DES = desmethyl linuron; please refer to Fig. 1 (appended) for structures.

Extraction of Label From Tissues

Liver- Attempts were made to extract radiolabel retained by livers using n-hexane, methanol/water, or protease. Extraction efficiencies of about 25-50% of the label retained in liver were reported for the three groups, but due to the low levels of radioactivity and the presence of lipids in the n-hexane fraction, no specific metabolites were identified. Group I livers were reported to contain one predominant nonpolar compound and at least three minor components. Group II livers were reported to contain many components which were nonpolar and similar to Group I. Male Group III livers were reported to contain at least three major and three minor "reasonably" nonpolar compounds. The livers of Group III females were also reported to contain "reasonably" nonpolar metabolites, however the number was not reported. One polar component (origin) was reported in the male and female group III livers. A small amount of radioactivity was extracted from the livers of Group III rats after protease treatment. Although the amount of label released was reportedly too small to characterize structure, the fact that any label was released after protease treatment may suggest covalent binding of label to liver proteins, or may reflect release from plasma albumin (see below).

Blood- Extraction of blood samples from group III (high dose) rats with ethyl acetate or methanol released only 5% and 2.5%, respectively, of the residual label contained in blood. Treatment with protease released 45% and 75% of the residual label in males and females, respectively. It was not clear whether this extraction was of packed cells or of whole blood. Therefore, the apparent significant release of label after protease treatment may have been due to binding of label to red cell proteins or to plasma albumin, which is well-known to bind many xenobiotics non-covalently.

Between 30-50% of residual label in kidney, gonads, or muscle was extractable by n-hexane or methanol/water. The amount of radiolabel was reportedly insufficient for chromatographic analysis.

Discussion

¹⁴C-Linuron (universal ring label), when administered orally (gavage) to male or female rats as a single dose of 24 mg/kg (Group I) or 400 mg/kg (Group III), or as a single dose of 24 mg/kg after 21 days of preconditioning with 100 ppm unlabeled compound in the diet (Group II) was rapidly absorbed, metabolized and excreted. The primary route of excretion was the urine (60-76%), and the feces (13-25%) was the secondary route of elimination. No ¹⁴CO₂ was detected, indicating that respiration was not a significant metabolic route for excretion. The degree of gastrointestinal absorption appears to be significant since the ratio of radioactivity found in the urine to that in the feces ranges from 2.4 to 5.

Biological half-lives of excretion were different between low dose (24 mg/kg) males and females 21 and 35 hours, respectively. The high dose group (400 mg/kg) had a half-life of approximately 55 hours with no apparent difference between sexes. This longer half-life in high dose rats may be due to a saturation of the metabolic pathway or effects on absorption from the gastrointestinal tract.

A large percentage (up to 60%) of excreted metabolites, urinary and fecal, were not identified. Therefore, in the opinion of this reviewer, speculation as to the metabolic pathway of linuron is premature. The major fecal metabolites that were identified were NOR-linuron (1-[3,4-dichlorophenyl]urea; up to 38% of label/fecal sample) and OH-NOR-linuron (4,5-dichloro-2-hydroxyphenylurea; up to 26% of label/fecal sample). OH-NOR linuron was the major urinary metabolite (up to 53% of label/urine sample). An apparent relationship between label excreted as "origin" and as OH-NOR linuron was noted in that the relative % excreted as origin decreased as the % excreted as OH-NOR increased.

There appeared to be some sex and/or dose group differences in fecal metabolites. Group I and II females appeared to form more NOR-linuron than did males at 24 and 48 hours. Group III animals excreted more label as NOR-linuron than Groups I or II, however no difference between the sexes was apparent. In contrast, more OH-NOR-linuron appeared to be excreted in the feces of groups I and II males than females or Group III males and females at 24 and possibly 48 hours. No UNII metabolite (unidentified) was apparently detected in the feces, in contrast to the urine.

For urinary metabolites, the origin contained as much as 51% of the excreted radioactivity, with Groups I and II appearing to excrete a greater percentage of extracted label in this form than did Group III animals. Also, a greater relative amount was excreted in this form at 6 hours in all groups as compared to later time points, with the exception of Group III females who apparently had no excretion of label in this form at 6 hours. Apparently no UNIII metabolite was detected in the urine of any of the test animals, in contrast to the feces. The UNII metabolite was detected in urine only for Groups I and III (but not II) rats and generally composed less than 10% of the excreted label. A possible sex difference (at 6 hours) in excretion of OH-desmethyl linuron and desmethyl linuron was reported, however this finding was not noted at later time points, and the small number of animals studied makes the significance of this observation unclear.

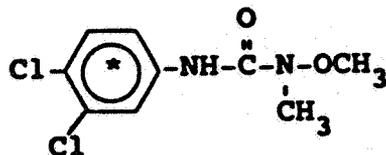
A concern was previously raised regarding possible contamination of technical linuron by dichloroaniline (DCA), hydroxy-dichloroaniline (OH-DCA), tetrachloroazobenzene (TCAB) and tetrachloroazoxybenzene (TCAOB). These compounds have been associated with numerous toxic effects. Although none of these compounds were identified in the submitted study, because of the large percentages of unidentified metabolites, their presence or absence should be considered tentative.

Less than 1% of the administered dose remained in the body at either 96 or 120 hours post-dosing, suggesting that linuron poses little hazard for bioaccumulation.

The testes, which were a target tissue for oncogenic effects in the 2-year rat chronic feeding study (benign testicular adenomas) did not appear to bioaccumulate radiolabel. However, there appeared to be a differential decrease of concentration in Group II males after preconditioning, which suggests a possible saturation of a compartment in the testes; the accumulation of label by other organs was not affected by preconditioning. As noted earlier, this difference may simply be the result of the small numbers of animals utilized. Blood, which is also an apparent target for linuron toxicity (sulf- and methemoglobinemia), released radioactivity upon treatment with protease, but this finding may have been due to binding of label to plasma albumin, which is well-known to bind many xenobiotics non-covalently.

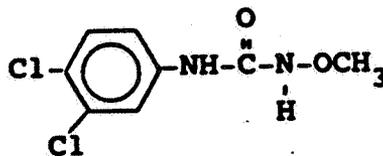
FIGURE 1

NAMES AND STRUCTURES OF LINURON AND RELATED COMPOUNDS



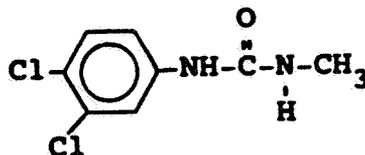
LIN (Linuron)

1-(3,4-dichlorophenyl)-3-methoxy-3-methylurea



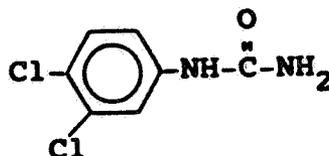
DESMETHYL

1-(3,4-dichlorophenyl)-3-methoxyurea



DESMETHOXY

1-(3,4-dichlorophenyl)-3-methylurea

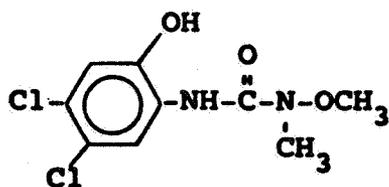


NOR

1-(3,4-chlorophenyl)urea

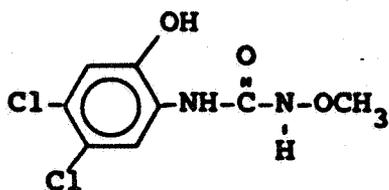
FIGURE 1 (CONTINUED)

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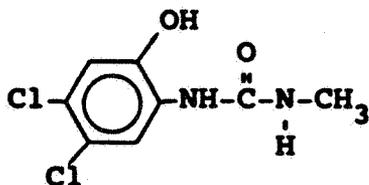
HO-LIN

1-(4,5-dichloro-2-hydroxyphenyl)-3-methoxy-3-methylurea



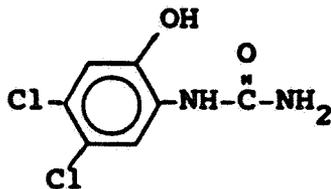
HO-DESMETHYL

1-(4,5-dichloro-2-hydroxyphenyl)-3-methoxyurea



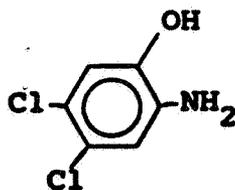
HO-DESMETHOXY

1-(4,5-dichloro-2-hydroxyphenyl)-3-methylurea



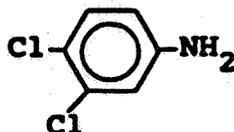
HO-NOR

4,5-dichloro-2-hydroxyphenylurea



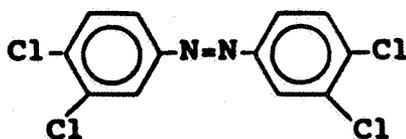
HO-DCA

4,5-dichloro-2-hydroxyaniline



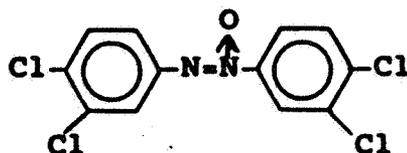
DCA

3,4-dichloroaniline



TCAB

3,4,3',4'-tetrachloroazobenzene



TCAOB

3,4,3',4'-tetrachloroazoxybenzene

* Denotes location of ¹⁴C-label.