difference can be attributed to the differences in half-lives observed. The consistency of these values at the various times in our study supports the concept that the kinetics of TCDD retention and elimination are adequately described by a single-compartment model.

A second method of calculating steady-state retention provides an estimate of TCDD absorption from the GI tract when compared to the values in Table VIII. At steady state, TCDD intake (eq 1) will equal TCDD excretion (kA_s). The total body burden is described by eq 2.

$$A_s = I/k \quad (2)$$

When the k values in Table VII were used, the A_s values for males and females were 17.2 and 21.7 times intake, respectively. These values are considerably higher than the values shown in Table VIII and imply that only 50–60% of the TCDD was absorbed. The results of Piper *et al.* (1973) suggest that 70% was absorbed in their study when TCDD was administered as a single dose in oil. It is not unreasonable to expect that TCDD incorporated in the feed is absorbed less efficiently than TCDD dissolved in oil.

These estimates implicitly assume that a number of parameters are constant, *i.e.*, concentration of TCDD in the diet, level of feed intake, body size, and body composition. In the "real world" none of these can be expected to be constant and a true steady state would never be reached. In addition, our values for TCDD were based on ^{14}C activity and assume that there was no metabolism. This is a valid assumption at this time but future studies may demonstrate that some of the activity was actually a metabolite.

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Metabolic Fate of Clopidol after Repeated Oral Administration to Rabbits

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After oral administration of single (16 mg/kg) or multiple doses (16 mg/kg per day) of [^{14}C]-3,5-dichloro-2,6-dimethyl-2,6-[^{14}C]pyridin-4-ol (clopidol) to rabbits most of the material was rapidly absorbed and almost exclusively excreted in the urine. Following repeated daily oral doses, no accumulation of radioactivity occurred in the tissues and no radioactivity was detected in the expired air. During a period of withdrawal after five daily doses, radioactivity was below the limits of detection in the tissues or plasma of rabbits killed later than 32 hr after administration of the last dose. Three major radioactive components were detected in the rabbit urine, two of these were identified by tlc and mass spectrometry as unchanged clopidol and 3,5-dichloro-2-hydroxymethyl-6-methylpyridin-4-ol accounting for a mean of 47 and 32%, respectively, of the urinary radioactivity. The third metabolite, accounting for a mean of 20% of the urinary radioactivity, was probably the *O*-glucuronide of the hydroxylated metabolite.

Clopidol, 3,5-dichloro-2,6-dimethylpyridin-4-ol, is widely used for the control of coccidiosis in chickens (Stevenson, 1965; Stock *et al.*, 1967) and its chemotherapy has been reviewed (Ryley and Betts, 1973). Smith and Watson (1969) showed that [^{36}Cl]clopidol was rapidly absorbed and excreted by rats after oral administration.The half-life of radioactivity in most tissues was about 10 hr and almost equal amounts were excreted in the urine and feces. The radioactivity excreted in the feces may have been due to unabsorbed clopidol or may have represented compounds excreted in the bile, but this was not determined. The metabolism of [^{36}Cl]clopidol in chickens has also been investigated (Smith, 1969) when the residues in tissues were identified as unchanged clopidol. As part of the safety evaluation for the use of this compound in other species we have studied its metabolic fate in rabbits after single and repeated doses.

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MATERIALS AND METHODS

3,5-Dichloro-2,6-dimethyl-2,6-[^{14}C]pyridin-4-ol, ([^{14}C]-clopidol) of specific activity 0.943 mCi/mmol, unlabeled clopidol, and reference compounds 3,5-dichloro-2-hydroxymethyl-6-methylpyridin-4-ol and 3,5-dichloro-2-carboxy-6-methylpyridin-4-ol were supplied by Dow Chemical Co., Kings Lynn, U.K. Thin-layer chromatography showed that [^{14}C]clopidol was >99% radiochemically pure.

Thin-Layer Chromatography. Thin-layer chromatography (tlc) was carried out on prelayered Kieselgel F_{254} plates (E. Merck A.G., Darmstadt, Germany) of layer thickness 0.25 or 2 mm. The solvent systems used and R_f values of reference compounds are shown in Table I.

^{14}C-Labeled metabolites were detected by autoradiography using Kodak Kodirex X-ray film. Radioactive areas of the gel were removed and counted in a Triton X-100 scin-

Table I. Tlc Solvent Systems Used and R_f Values of Reference Compounds

Solvent system (v/v)	Clopidol	3,5-Dichloro-2-hydroxymethyl-6-methylpyridin-4-ol	3,5-Dichloro-2-carboxy-6-methylpyridin-4-ol
(a) Butan-1-ol–benzene–30% NH_3 soln. (9:2:2)	0.44	0.30	0.00
(b) Butan-1-ol–acetic acid–water (2:1:1)	0.73	0.69	0.59
(c) Ethanol–water–30% NH_3 soln. (30:10:1)	0.73	0.70	0.67
(d) Ethyl acetate–formic acid–water (60:5:35 organic phase)	0.42	0.35	0.07
(e) Chloroform–ethanol–acetic acid (16:4:1)	0.70	0.51	0.05

Table II. Dosing Schedule Followed for the Repeated Administration of Clopidol to Rabbits

Rabbit no.	No. of doses at 24-hr intervals	Time sacrificed: After first dose, hr	Time sacrificed: After last dose, hr	Total dose of clopidol administered, mg
1	1	0	0	40
2	1	24	24	40
3	2	48	24	80
4	5	96	0	200
5	5	112	16	200
6	5	128	32	200
7	5	160	64	200
8	5	224	128	200

tillator gel (Chasseaud *et al.*, 1972). This technique gave recoveries of radioactivity exceeding 95%. Reference compounds were detected by quenching of fluorescence at 254 nm.

Animal Experiments. The dosage form was prepared by thoroughly mixing [^{14}C]clopidol (227 mg) and nonradioactive clopidol (1.083 g) in 1% (w/v) carboxymethylcellulose (68 ml). This preparation was stirred regularly to ensure a homogeneous suspension.

Eight adult New Zealand White rabbits (body weight *ca.* 2.5 kg) were used. During the dosing period the animals were housed singly in stainless steel metabolism cages which facilitated the separate collection of urine and feces. The animals were allowed unrestricted access to water and a standard pelleted diet. Urine was collected in receivers cooled in Dry Ice, and all collected excreta were stored at −20° until taken for analysis. The expired CO_2 from one animal was trapped in 20% (v/v) ethanolamine–2-ethoxyethanol.

Each rabbit was dosed by oral intubation with 2 ml of [^{14}C]clopidol suspension at a dose level of 16 mg/kg, once each day for up to 5 days. Rabbits were dosed and sacrificed according to the schedule shown in Table II.

Urine was collected in receivers cooled in Dry Ice as a combined specimen from each rabbit during the period from administration of the first dose to the time of sacrifice. Feces were collected from each animal as a combined specimen during the same period. After sacrifice, the liver, kidneys, bladder contents, gastrointestinal tract and contents, and samples of muscle and blood were removed from the carcasses. Tissue samples, carcasses, and collected excreta were stored at −20°.

Preparation of Samples and Measurement of Radioactivity. Feces, finely minced carcasses, and tissues were macerated with methanol. After centrifugation, radioactivity was measured in both the supernatant and in the residue.

A Philips Liquid Scintillation Analyser was used for measurement of radioactivity using external standard channels ratio quench correction. Samples of urine, plasma, solvent extracts of feces, tissues, and whole carcasses, the contents of the expired air traps, and the cage washings were mixed with a Triton X-100 based scintillator system (Patterson and Greene, 1965). Samples of the residue from the solvent extractions of feces, carcasses, and tissues were oxidized by a modification of the plastic bag technique (Lewis, 1972) and the combustion products absorbed into a β-phenylethylamine-based scintillator system (Dobbs, 1963).

Detection and Measurement of Urine Metabolites. For the detection and measurement of metabolites, samples of urine were applied directly to tlc plates (0.25 mm) and chromatographed in solvents a to e. In the deconjugation studies, portions of urine were incubated with a β-glucuronidase preparation (type H2, Sigma Chemical Co., St. Louis, Mo.) or arylsulfatase preparation (type III, Sigma Chemical Co.) in 0.2 *M* sodium acetate buffer (pH 5.5) at 37° for 18 hr. The incubated samples were subjected to tlc in solvents a and c. Urine samples were hydrolyzed by heating with equal volumes of 2 *M* HCl at 100° for 16 hr and the hydrolysates were examined by tlc.

Separation and Characterization of Metabolites. Portions of urine were applied to Amberlite XAD-2 resin columns (The Rohm and Haas Co., Philadelphia, Pa.) by the method of Fujimoto and Haarstad (1969). The column was washed with water and eluted with methanol. The aqueous wash contained 20% of the applied radioactivity and the methanol eluate 80%. The aqueous solution was discarded, and the methanol eluate was concentrated *in vacuo* and applied to tlc plates (2 mm) developed in solvent b. The two separated radioactive areas were eluted with 1 *M* NaOH. The resulting solutions were neutralized with 2 *M* HCl and the radioactive precipitate separated and washed with distilled water.

Samples of urine were also concentrated *in vacuo* at 40° in a rotary film evaporator and filtered and the filtrate applied to tlc plates (2 mm). The minor and most polar metabolite was eluted with 1 *M* NaOH–methanol (1:1, v/v). Methanol was removed in a rotary film evaporator, and the remaining solution was neutralized with 2 *M* HCl. The precipitate that formed did not contain radioactivity and was discarded. To the remainder was added 0.2 *M* sodium acetate buffer (pH 5.5) and the resulting solution was incubated at 37° for 3 hr with β-glucuronidase. The incubated solution was subjected to tlc in solvent system b.

Two urinary components were isolated and methylated with diazomethane in methanol and the derivatives subjected to mass spectrometry.

Table III. Radioactivity Present in the Excreta and Tissues of Rabbits which Received up to Five Daily Oral Doses of [^{14}C]Clopidol (16 mg/kg) (Expressed as Per Cent Total Dose Received)

	Rabbit no.							
	1	2	3	4	5	6	7	8
No. of daily doses	1	1	2	5	5	5	5	5
Time of sacrifice								
hr after first dose	0	24	48	96	112	128	160	224
hr after last dose	0	24	24	0	16	32	64	128
Radioact. present								
Voided urine	NS[a]	97.9	83.7	81.6	105.3	91.1	95.9	102.6
Bladder contents	<0.002	0.69	13.25	0.03	1.55	0.14	0.004	0.004
Feces	NS	0.5	1.9	1.7	2.7	4.1	3.0	1.3
Expired air			<0.05					
Gastrointestinal tract and contents	101.04	0.56	0.13	19.62	0.21	0.11	0.02	<0.005
Liver	<0.005	0.07	0.04	0.002	0.07	<0.001	<0.001	<0.001
Kidney	<0.005	0.02	0.01	0.002	0.03	<0.001	<0.001	<0.001
Muscle (in ~100-g samples)	<0.01	<0.01	<0.005	<0.002	0.01	<0.002	<0.002	<0.002
Remaining carcass	<0.05	0.36	0.24	0.05	0.65	0.08	<0.01	<0.01
Total recovery	101.0	100.2	99.3	103.0	110.5	95.5	98.9	103.9

[a] NS = no sample.

Table IV. Concentrations of Radioactivity in Tissues of Rabbits during and after Withdrawal from Five Daily Oral Doses of [^{14}C]Clopidol (16 mg/kg), Expressed as ppm Clopidol Equivalents

	Rabbit no.							
	1	2	3	4	5	6	7[a]	8[a]
Total dose, mg	40	40	80	200	200	200	200	200
Time of sacrifice, hr after last dose	0	24	24	0	16	32	64	128
Concentrations of radioact. present								
Liver	<0.01	0.34	0.28	0.04	1.36	<0.01	<0.01	<0.01
Kidney	<0.04	0.50	0.40	0.18	2.26	<0.04	<0.04	<0.04
Muscle	<0.04	<0.04	<0.04	<0.04	0.39	<0.04	<0.04	<0.04
Plasma	<0.01	0.81	0.74	0.25	2.80	0.03	<0.01	<0.01
Remaining carcass	<0.01	0.06	0.07	0.04	0.52	0.06	<0.01	<0.01

[a] Tissue residues below the limits of detection in these animals.

Mass Spectrometry. Mass spectra of isolated metabolites were recorded on a Hitachi Perkin-Elmer RMS-4 instrument (Perkin-Elmer Limited, Beaconsfield, Bucks, U.K.) operating at an ionizing potential of 70 eV and an ionizing current of approximately 100 μA. Samples were introduced by the direct insertion probe at a chamber temperature of 180–220°. Spectra were normalized by processing on punched paper tape in an Elliott 903 computer; histograms were produced on an IBM 360/44 using a computer-controlled graph plotter.

RESULTS

Excretion and Retention of Radioactivity. Most of an oral dose of [^{14}C]clopidol administered to rabbits was rapidly absorbed and excreted in the urine (Table III). In the case of rabbit no. 2, killed 24 hr after a single dose, and rabbit no. 3, killed 24 hr after the second of two daily doses, 99 and 97% of the total respective doses were recovered from the urine and bladder contents. In rabbit no. 4, which was killed immediately after the last of the five daily doses, 20% of the total dose administered was present in the gastrointestinal tract, and the remaining 80% of the dose had been excreted in the urine (Table III). In this rabbit, 20% of the total dose is equivalent to all of the final dose, and the preceding doses had therefore already been absorbed and excreted in the urine.

Four other rabbits (no. 5–8) were killed at different times after receiving the last of five doses (Table III). Most of the dose administered to rabbit no. 5, killed 16 hr after administration of the final dose, was recovered in the urine, feces, and bladder contents. Less than 1% of the total dose administered remained in this animal's carcass at the time of sacrifice. Only 0.08% of the total dose remained in the carcass of rabbit no. 6, killed 32 hr after administration of the last dose. No radioactivity was detected in the expired air of one rabbit (no. 3, Table III) indicating the clopidol had been radiolabeled in metabolically stable positions.

Tissue Residues. The limits of detection in tissues were 0.01–0.04 ppm clopidol equivalents (Table IV). Concentrations of clopidol and its metabolites present in the tissues of animals killed either during the dosing or withdrawal periods were low (Tables III and IV). Concentrations of radioactivity expressed as clopidol equivalents were greater in the plasma than in the tissues showing that no selective tissue uptake occurred (Table IV). No accumulation of radioactivity was apparent in those animals which had received five daily doses of clopidol. Of

Table V. Proportion of Radioactive Components Excreted in the Urine of Rabbits after Oral Administration of [^{14}C]Clopidol (Expressed as Per Cent Total Urinary Radioactivity)

	Rabbit no.							
	2	3	4	5	6	7	8	Mean
UC1	14.4	19.4	27.5	18.4	29.3	20.0	16.8	20.8
UC2	23.7	29.4	38.0	27.5	17.4	65.7	23.7	32.2
UC3	61.9	51.2	34.5	54.1	53.4	14.3	59.5	46.9

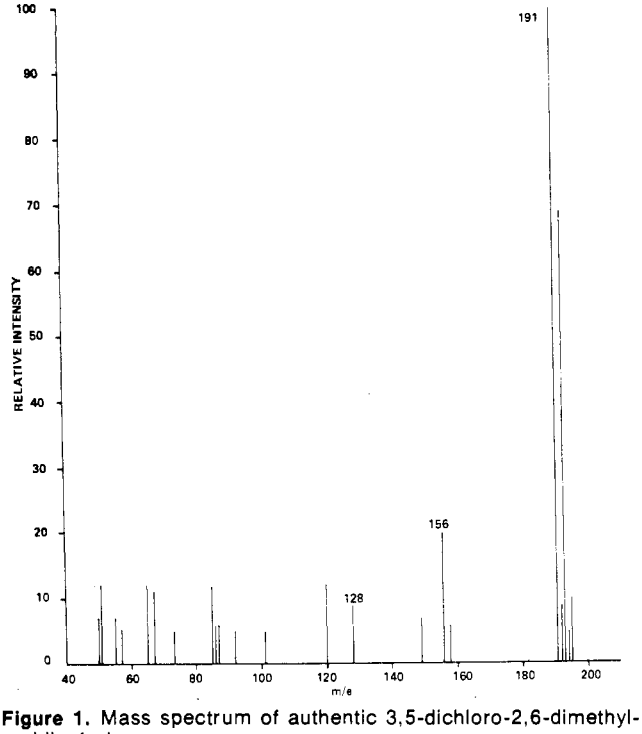


Figure 1. Mass spectrum of authentic 3,5-dichloro-2,6-dimethylpyridin-4-ol.

the animals killed at intervals after the last of five daily doses, the radioactivity was below the limits of detection in the muscle, liver, and kidneys of animals killed 32 hr after administration or later, although low concentrations were detected in one plasma sample and the carcass from rabbit no. 6. Based on the carcass or plasma levels, the biological half-life of clopidol and its metabolites was calculated to be about 3 hr.

Detection and Identification of Metabolites. Three major radioactive components were separated by tlc of rabbit urine in five different solvent systems. These components were designated UC1, UC2, and UC3, respectively. When added to urine, reference standards of clopidol and 3,5-dichloro-2-hydroxymethyl-6-methylpyridin-4-ol were chromatographically identical with radioactive urinary components UC3 and UC2, respectively. The proportions of each major component present in the urines of different rabbits are shown in Table V. The results are those obtained from solvent a. The R_f values of UC3 were similar in all solvent systems to those of unchanged clopidol; this component could not be resolved from authentic [^{14}C]clopidol added to urine. Some individual variations are apparent in the proportions of urinary radioactivity which were excreted as each component. Rabbits 6 and 7 excreted 17.4 and 65.7% of the total urinary radioactivity as UC2. A fourth minor urinary component (R_f 0.65 in solvent system b) was also present in rabbit urine and was chromatographically different from clopidol, 3,5-dichloro-2-hydroxymethyl-6-methylpyridin-4-ol, and 3,5-dichloro-

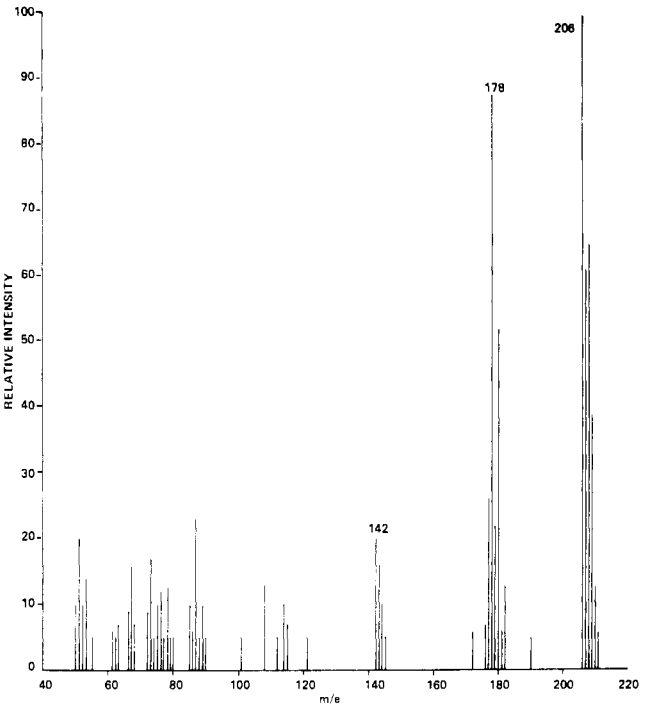


Figure 2. Mass spectrum of authentic 3,5-dichloro-2-hydroxymethyl-6-methylpyridin-4-ol.

2-carboxy-6-methylpyridin-4-ol in solvent systems b and e. This compound represented less than 2% of the urinary radioactivity.

Incubation of rabbit urine with β-glucuronidase converted UC1 to a less polar component having the same R_f value as either UC2 or UC3 on tlc plates developed in solvents a or b. UC1 was not altered by treatment with arylsulfatase or acid hydrolysis at 100°. On the basis of this evidence, UC1 may be an acid-stable glucuronide conjugate of UC2 or UC3.

UC1 was isolated by tlc of freeze-dried rabbit urine and a sample was incubated with β-glucuronidase. The incubated sample contained a single component with chromatographic properties identical with UC2. These results suggest that UC1 was probably a glucuronide conjugate of UC2.

To obtain material suitable for mass spectrometry, rabbit urine was passed through a column of Amberlite XAD-2 resin. UC1 appeared in the aqueous wash from this column and UC2 and UC3 in the methanolic eluate. Tlc of the methanolic eluate in solvent system a partially separated UC2 and UC3. These components were extracted from the silica gel with NaOH solution. Addition of HCl precipitated UC2 and UC3, which were separated by centrifugation, washed with water, dried, and subjected to mass spectrometry.

The mass spectra of the authentic reference compounds, clopidol and the hydroxylated derivative, are shown in Figures 1 and 2, respectively. Both of these compounds give very characteristic molecular ions showing the expected multiplet due to the natural abundance of the chlorine isotopes (^{35}Cl:^{37}Cl, 3:1). These molecular ions and other fragments containing two chlorine atoms consist of multiplets with peaks at M, M + 2, and M + 4 in the ratios 9:6:1, respectively.

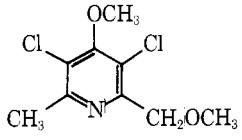
The mass spectrum of clopidol shows a molecular ion, also the base peak, at m/e 191 and fragment ions at m/e 156 and 128 resulting from successive loss of a chlorine and carbon monoxide, respectively (Figure 1). The mass spectrum of the hydroxylated derivative (Figure 2) shows a molecular ion at m/e 207 but a more intense P − 1 ion at m/e 206, the base peak. The major fragmentation of m/e 206 involves loss of carbon monoxide to give m/e 178

and loss of a chlorine to give m/e 143. The fragment ions at m/e 177 and 142 could arise from loss of formaldehyde and a chlorine from the molecular ion.

The mass spectrum of impure UC2 (Figure 3) showed that it was contaminated with some clopidol (UC3). However, the characteristic pattern in the molecular ion region and the fragment ions at m/e 178 and 142 were identical with those present in the spectrum of the authentic hydroxylated derivative.

The mass spectrum of UC3 showed a molecular ion at m/e 191, identical with clopidol. But none of the other much less intense fragment ions observed in the mass spectrum of clopidol could be identified due to the high background arising from biological contaminants.

Methylation of UC2 and UC3 with diazomethane gave less polar derivatives. The mass spectrum of methylated UC2 showed a molecular ion at m/e 235 consistent with the formation of a dimethyl ether (1) and the mass spectrum of methylated UC3 showed a molecular ion at m/e 205 consistent with a methyl ether of clopidol (2).



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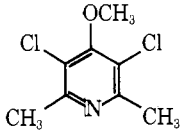

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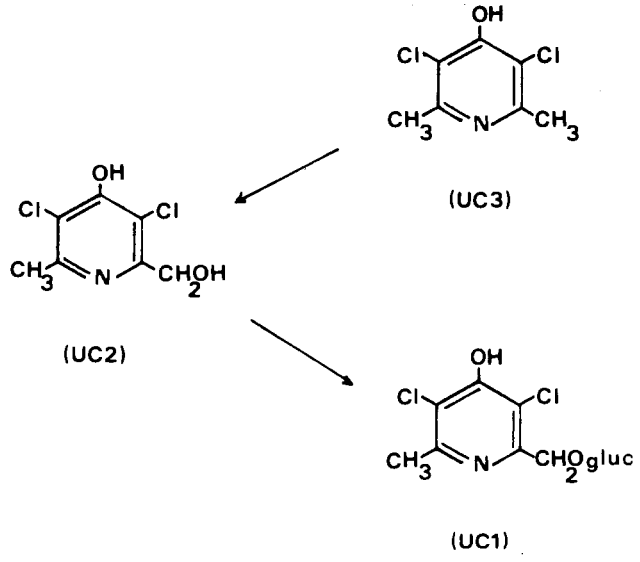
DISCUSSION

Clopidol is a relatively nonpolar compound although it is very insoluble in most solvents (Smith, 1969), the greatest solubility being in strongly acidic or basic media. [^{14}C]Clopidol was rapidly and quantitatively absorbed by rabbits after oral doses and was rapidly and completely excreted in the urine. After repeated doses no tissue accumulation of radioactivity was apparent, and most of the radioactivity administered daily was excreted in the urine, before administration of the next dose.

Part of an oral dose of [^{14}C]clopidol underwent biotransformation in rabbits. Of the radioactivity excreted in the urine, a mean of 47% was associated with a component corresponding to unchanged clopidol, while 3,5-dichloro-2-hydroxymethyl-6-methylpyridin-4-ol and its corresponding glucuronide accounted for 32 and 21% of the urinary radioactivity, respectively. The polarity on tlc of the minor component present in rabbit urine suggests that it may be 3,5-dichloro-2,6-dihydroxymethylpyridin-4-ol. The proposed metabolic pathway of clopidol is outlined in Scheme I.

Scheme I. Probable Biotransformation of Clopidol



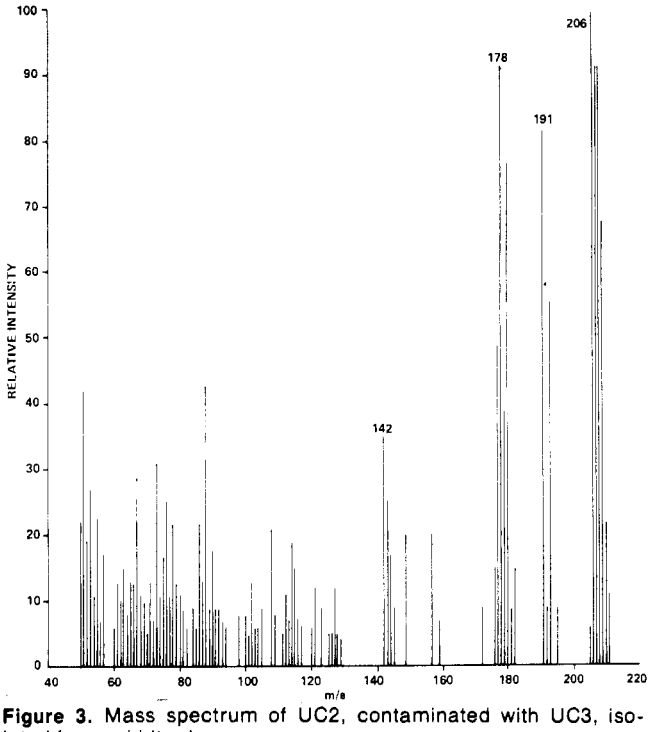
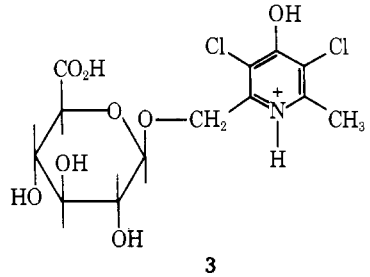


Figure 3. Mass spectrum of UC2, contaminated with UC3, isolated from rabbit urine.

The conjugate is probably an ether glucuronide linked *via* the primary hydroxyl group rather than through the phenolic group as clopidol itself does not appear to form a glucuronide. The acid stability of the postulated ether-glucuronide may be explained on electronic grounds. The protonated form of the glucuronide (3) will be the predominant species present in acidic solution and the aglycone will exert a strong electron-withdrawing influence on the glycosidic link. This will reduce the susceptibility to protonation and subsequent hydrolytic cleavage.



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On a physicochemical basis it would be expected that the biotransformation products of clopidol would be more rapidly excreted than clopidol itself and unlikely to accumulate in the rabbit. Both metabolites, especially the glucuronide, should be less lipid soluble than clopidol and consequently the renal clearances should also be higher.

Smith and Watson (1969) have shown that after administration of single oral doses (10 mg/kg) of [^{36}Cl]clopidol to rats 75–80% of the radioactivity was excreted in the urine and 20–25% in the feces, mainly within 24 hr. The half-life of radioactivity in the major tissues was 10 hr and preliminary investigations showed that the residual tissue radioactivity was unchanged clopidol.

In a similar experiment (Smith, 1969) when [^{36}Cl]clopidol was administered to chickens at a level of 0.0125% in the diet, 90% of the tissue radioactivity was eliminated within 2 days after withdrawal of the compound. The nature of the radioactivity in tissues was unambiguously identified as clopidol.

This study has shown that the elimination of oral doses of clopidol in another species, the rabbit, is similar to that

in the chicken and rat. However, the tissue levels of radioactivity in the rabbit, representing clopidol and metabolites, were lower than those in either the chicken or rat, and were below the limits of detection in all tissue 32 hr after administration of the last dose.

The nature of the radioactivity excreted by the rat and chicken has not been investigated, but this study has shown that in the rabbit, the major pathway of metabolism involves hydroxylation and then glucuronidation of the resulting alcohol.

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Carbaryl Penetration into and Metabolism by Alfalfa Leafcutting Bees, *Megachile pacifica*

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Carbaryl metabolism by and penetration into adult alfalfa leafcutting bees, *Megachile pacifica* (Panzer) (= *rotundata* Fabr.), were studied in relation to sex, age, and exposure to piperonyl butoxide. Unchanged carbaryl was a principal compound isolated from the bees after 4 hr, being more prominent in 4-day-old males (48% of extractable metabolites) and less prominent in 4-day-old females (22%) and 1-day-old males (26%). 1-Naphthol, 5,6-dihydro-5,6-dihydroxycarbaryl, 5-hydroxycarbaryl, and carbaryl were recovered from acid hydrolysis of water-soluble conjugates. Two minor organosoluble metabolites were not identified as were none of the conjugating moieties. Carbaryl persistence in the bees was closely related to carbaryl susceptibility. The results demonstrated that the differences in carbaryl LD_{50} values with and without piperonyl butoxide treatment are more meaningful than the quotient of these values.

Lee and Brindley (1975) have reported that adults of the alfalfa leafcutting bee, *Megachile pacifica* (Panzer) (= *rotundata*), are particularly tolerant to carbaryl. The carbaryl tolerance was related to sex and age. Dose–mortality studies showed the carbaryl LD_{50} to be similar for 1-day-old males and 1- and 4-day-old females (240, 245, and 262 μg/g, respectively). Four-day-old males, however, were much more susceptible having an LD_{50} value of 51 μg/g.

Estimations of carbaryl–piperonyl butoxide synergist ratios did not correlate well with the LD_{50} data. Brattsten and Metcalf (1970, 1973) have used the quotient of lethal dose concentrations of carbaryl alone divided by the lethal dose concentrations of carbaryl with the synergist piperonyl butoxide to provide carbaryl–piperonyl butoxide synergist ratios. These ratios provide convenient values for estimating and ranking microsomal oxidase activity in various insects. Lee and Brindley (1975), however, have suggested that use of such a synergist ratio can provide misleading results, and that the difference in the lethal dose levels may be a better *in vivo* estimate of carbaryl detoxication potential.

Treatment of alfalfa leafcutting bees with piperonyl butoxide prior to carbaryl treatment reduced the LD_{50} values such that the quotients (synergist ratios) were 12, 53, and 15 for 1-day-old males, 4-day-old males, and 4-day-old females, respectively (Lee and Brindley, 1975).

Therefore, this study was designed to study carbaryl metabolism and penetration in adult male (1 and 4 days old) and female (4 days old) alfalfa leafcutting bees and to elucidate the effects of piperonyl butoxide on the metabolism of carbaryl.

The alfalfa leafcutting bee is of great importance and efficiency in pollination of alfalfa for seed. Alfalfa seed acreage in 1972 was 378,100 acres, with an estimated seed value of more than $46,000,000 (United States Department of Agriculture, 1973). Idaho, Utah, Nevada, Washington, Oregon, and California had only 48% of this acreage but more than 85% of the revenues and average yields of 480 pounds per acre. The remaining seven states listed as alfalfa seed production areas averaged 90 pounds of seed per acre. The yield difference is due to several factors, but especially due to the presence of effective alfalfa leafcutting bee management.

MATERIALS AND METHODS

Insect and chemical sources, insect maintenance, and chemical applications were as described by Lee and Brindley (1975) except that carbaryl doses were made at 1 μg/bee under light CO_2 anaesthesia in all cases.

1-Naphthyl *N*-methyl-^{14}C-carbamate (methyl labeled) with a specific activity of 26.4 mCi/mmol and 1-naphthol-*1*-^{14}C with a specific activity of 15.2 mCi/mmol were purchased from the Amersham–Searle Corp., Arlington Heights, Ill. Both had radiochemical purities of more than 98%, constituted one spot in our tlc system, and were not further purified. The 1-naphthol-*1*-^{14}C was synthesized to ring-labeled carbaryl by reaction with an excess of methyl isocyanate (Dorough and Casida, 1964). Thin-layer chromatography (tlc) plates coated with a 1-mm thick silica

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