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Metabolism of Norflurazon by Rats

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Within 4 days of dosage with 2 and 110 mg/kg of [4,5-^{14}C-*pyridazinyl*]norflurazon, rats excreted 19–28 and 65–80% of the applied radiocarbon in the urine and feces, respectively. Four days posttreatment, the residues remaining in the carcass were minimal (<1% applied dose). Norflurazon was thoroughly degraded as evidenced by the paucity of parent compound in excrement (≤2% of applied dose). Nine metabolites were identified from the urine and feces, arising predominantly by two major pathways: N-demethylation and reaction with glutathione. Although norflurazon was degraded to numerous metabolites, only one (sulfoxide **3**) contributed more than 10% of the applied radioactivity. The other identified metabolites each represented 1–2% of the applied dose.

Norflurazon is a fluorinated pyridazinone herbicide registered under the trade names Zorial, Solicam, and Evital. It is utilized as a preemergent herbicide to selectively control annual grasses and broadleaf weeds in numerous commercial crops. Norflurazon is employed also for noncrop uses such as at airports, storage areas, and rights of way. This herbicide is absorbed by the roots of germinating weeds and is translocated to the growing areas where, in susceptible species, it inhibits carotenoid biosynthesis, resulting in chlorophyll photodegradation (Sandmann and Böger, 1982). Norflurazon is also an inhibitor of fatty acid desaturation in both plants and rat liver cells (Hagve et al., 1985). When emerging from the treated soil, the weed seedlings are white, become necrotic, and soon die.

This work was undertaken to define the fate of norflurazon in a mammalian model, the rat. Although the metabolism of norflurazon was studied more than a decade ago in rats (Karapally, 1974), recent advances in analytical methodology and instrumentation allowed us to identify several new metabolites. We now report the metabolic fate of norflurazon in rats.

EXPERIMENTAL SECTION

Analytical Methods. Reversed-phase liquid chromatography (LC) was performed with Spectra-Physics instruments (Models 8000A and 8700): ultraviolet detection at 254 nm; 10-μm Li-Chrosorb RP-8 column, 25 × 0.46 cm; elution at 1.6 mL/min. The following solvent systems of acetonitrile or methanol and a constant 0.1% trifluoroacetic acid were utilized (all gradients linear): SS 1, gradient 15–30% acetonitrile over 5 min, isocratic at 30% for 15 min, gradient 30–40% over 5 min, isocratic at 40% for 5 min, gradient 40–90% over 5 min, hold at 90% for 5 min; SS 2, gradient 30–35% methanol over 5 min, isocratic at 35% for 15 min, gradient 35–50% over 15 min, gradient 50–90% over 5 min, hold at 90% for 5 min; SS 3, gradient 15–22% acetonitrile over 5 min, isocratic at 22% for 15 min, gradient 22–40% over 5 min, gradient 40–90% over 5 min, hold at 90% for 5 min. Analysis by thin-layer chromatography (TLC) employed precoated silica gel GF plates (Analtech, 1000 μm) with development in the following mobile phases: SS 4, ethyl acetate; SS 5, ethyl acetate–hexane, 4:1; SS 6, chloroform–methanol, 5:1.

Radiocarbon was quantified by liquid scintillation counting (LSC, Packard Tri-Carb Models 4430 and 2425C) and by radiochromatography scanning (Bioscan System 200-HP).

Urine and feces were each extracted with a Polytron homogenizer (Brinkmann). After methylation (CH_2N_2), selected urinary and fecal extract samples were assayed by TLC, which involved cospotting the samples with appropriate authentic standards (visualized under UV light by quenching of fluorescence) followed by radiochromatographic scanning. The TLC zones were then scraped and eluted (methanol), and the ^{14}C was quantified by LSC. The radicarbon in certain zones was analyzed further by LC with coinjection of an aliquot plus selected authentic standards and collection of timed eluate fractions followed by LSC. Unextractable ^{14}C residues were combusted to $^{14}CO_2$, which was then trapped in scintillation fluid (Biological oxidizer, Model OX-300; Carbon 14 Cocktail; Harvey Instrument Co.) and quantified by LSC.

Mass spectra were obtained with a Hewlett-Packard instrument (Model 5985A) in the electron-impact (EI), chemical ionization (CI with CH_4), and fast atom bombardment (FAB) modes.

Synthetic Standards. The following authentic standards were supplied by Sandoz Ltd.: 4-chloro-5-(methylamino)-2-[3-(trifluoromethyl)phenyl]-3(2*H*)-pyridazinone (norflurazon, **1**); 4-chloro-5-amino-2-[3-(trifluoromethyl)phenyl]-3(2*H*)-pyridazinone (desmethylnorflurazon, **2**).

Radiosynthesis. [4,5-^{14}C-*pyridazinyl*]Norflurazon (96.5% pure, 2.85 mCi/mmol specific activity) was obtained from Sandoz Ltd. Additional purification of the [^{14}C]norflurazon (to 98.8% purity) was effected at Zoecon (TLC, SS 4).

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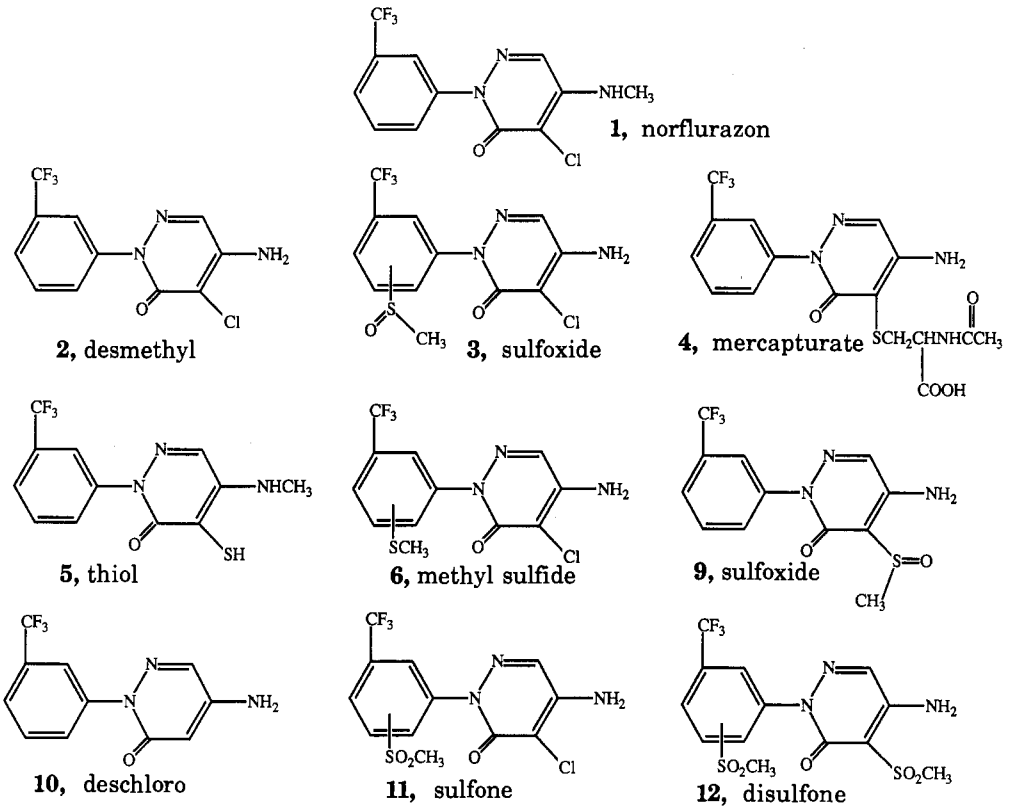

Figure 1. Structures of norflurazon and its metabolites.

Treatment. Healthy female rats (Sprague–Dawley, albino, ca. 200 g) were acclimated for at least 1 week prior to dosing. Four different groups of rats were dosed with [^{14}C]norflurazon as follows: single oral dose at 2 mg/kg (4 μCi) by gavage in corn oil (group 1, five animals); single oral dose at 110 mg/kg (4 or 21 μCi) by gavage in corn oil (group 2, five animals); single oral dose at 2 mg/kg (4 μCi) by gavage in corn oil within 24 h after continuous feeding of unlabeled norflurazon at 2 mg/kg in chow for 14 days (group 3, five animals); intravenous dose at 2 mg/kg (3 μCi) in physiological saline–ethanol (1:1, 200 μL; group 4, two animals). After treatment, each rat was immediately housed in an all-glass metabolism chamber that automatically separated urine and feces. In addition, one rat was monitored for expired radiolabeled organic volatiles, including $^{14}CO_2$. All rats were allowed free access to food (Purina rat chow) and water. Four days posttreatment, the animals were sacrificed and selected tissues removed for subsequent analysis.

Analysis of Urine. Certain urinary metabolites (**3–5**) were isolated from the urine of a rat (day 0–1) dosed at 110 mg/kg (single oral) in order to make structural assignments by spectral methods (Figure 1). The isolation of these metabolites (after methylation with CH_2N_2) was accomplished by TLC and LC assays as described below in detail. With this chromatographic information (Table I) as a comparison, the presence of metabolites **3–5** in the urine (after methylation) from dose groups 1, 3, and 4 was then estimated based on TLC (SS 4) and LC (SS 1) migration patterns. The amount of norflurazon and desmethylnorflurazon in the urine (all dose groups) was determined by coincident mobility with authentic standards upon TLC (SS 4) and by coelution with authentic standards upon LC (SS 1).

The first step in isolating urinary residues for spectral analysis involved fractionation of urine after methylation (day 0–1, dose group 2) by preparative TLC (SS 4) into four zones. Each zone was investigated in more detail by LC (SS 1). LC analysis of the TLC origin zone (R_f 0.0–0.11) yielded two major radioactive regions purified further by LC (SS 3 and then SS 2) to yield polar metabolites that could not be identified.

After isolation by TLC (R_f 0.11–0.32, SS 4) and LC (SS 1), metabolites **3** and **4** were characterized by mass spectrometry (Table II). Sulfoxide **3** reacted with acetyl chloride to give derivatives **6** and **7** in equal yields (Figure 2). Derivative **6** resulted from reduction of **3** to its methyl sulfide [cf. Numata and Oae (1973)], which was identified from mass spectral data. Derivative **7** contained both an acetylated amino group and a reduced sulfoxide as compared to **3**. The methyl ester of mercapturate **4** was converted to its *N*-acetyl derivative **8** by reaction with acetyl chloride. Thiol **5** was purified by TLC (R_f 0.54–0.80, SS 4) and LC (SS 1) prior to spectral analysis.

Table I. Mobility of Norflurazon and Its Metabolites

		k' (LC)		R_f (TLC)[a]	
metabolite	no.	SS 1	SS 2	SS 4	SS 5
norflurazon	1	8.0	15.7		0.46–0.64
desmethyl	2	6.0	12.7		0.46–0.64
sulfoxide	3	3.9	8.9	0.23	0.26
mercapturate	4	6.0		0.23	
thiol	5	8.0		0.63	
methyl sulfide	6	6.4	7.0		0.46–0.64
sulfoxide	9	5.2			0.40
deschloro	10	5.1			0.46–0.64
sulfone	11	3.9	6.0		0.46–0.64
disulfone	12	3.9	4.9		0.46–0.64

[a] The compositions of the solvent systems (SS 1–5) are given in the text.

Analysis of Feces. The feces were extracted (methanol, 3×), and selected samples of the resulting extracts and residual solids were analyzed in detail. The most abundant fecal ^{14}C residues (**1–4, 6, 9–12**) were isolated from the fecal extract (methylated with CH_2N_2) of a rat dosed at 110 mg/kg so that their structures could be determined by mass spectrometry. The specifics of these isolations are described below. By knowing R_f and k' values (from TLC and LC analysis) for the isolated metabolites from group 2 rats, the presence of these same metabolites in the fecal samples (after methylation) from groups 1, 3, and 4 could be determined by comparison. For norflurazon and desmethylnorflurazon, their abundance was also established by coincident mobility with authentic standards.

The fecal extract (day 0–1, methylated) from a rat dosed with [^{14}C]norflurazon at 110 mg/kg was examined thoroughly to isolate the most abundant residues for structural identification. Initial purification by column chromatography (Kieselgel 60, 70–230 mesh) separated the nonpolar fraction (ethyl acetate eluent) from the relatively polar fraction (methanol eluent). The polar fraction

Table II. Mass Spectral Data for Urinary Metabolites and Derivatives

metabolite	m/z (relative intensity)
sulfoxide 3	EI, 336 (12, M − CH_3), 290 (30), 288 (100), 261 (8), 234 (6), 206 (5), 145 (7), 88 (46); CI, 392 (5, M + C_3H_5), 380 (14, M + C_2H_5), 354 (35, M + H for ^{37}Cl), 352 (100, M + H for ^{35}Cl), 336 (4, M − CH_3), 332 (3, M − F), 288 (5, M − $SOCH_3$), 269 (13)
mercapturate 4 (methyl ester)	CI, 471 (2, M + C_3H_5), 459 (3, M + C_2H_5), 431 (45, M + H), 411 (7, M − F), 302 (12), 288 (24), 256 (85), 146 (64), 144 (100)
thiol 5	EI, 301 (68, M^+), 282 (8, M − F), 268 (100, M − SH), 204 (6), 145 (44), 100 (55); CI, 342 (4, M + C_3H_5), 330 (16, M + C_2H_5), 302 (100, M + H), 282 (18, M − F)
methyl sulfide 6	CI, 376 (3, M + C_3H_5), 364 (11, M + C_2H_5), 338 (35, M + H for ^{37}Cl), 336 (100, M + H for ^{35}Cl), 316 (38, M − F), 286 (25), 266 (9); EI, 335 (3, M^+), 306 (6), 288 (100), 261 (6), 234 (4), 204 (7), 88 (10)
7 (acetyl 6)	EI, 379 (3, M^+ for ^{37}Cl), 377 (9, M^+ for ^{35}Cl), 358 (5, M − F), 330 (86, M − SCH_3), 313 (26), 288 (100), 271 (12), 204 (9), 145 (4), 88 (8); CI, 418 (4, M + C_3H_5), 406 (9, M + C_2H_5), 380 (37, M + H for ^{37}Cl), 378 (100, M + H for ^{35}Cl), 358 (29, M − F), 336 (18, M − CH_2CO), 279 (8), 205 (21)
8 (acetyl 4, methyl ester)	CI, 501 (1, M + C_2H_5), 473 (8, M + H), 453 (3, M − F), 431 (6, M + H − CH_2CO), 330 (13), 298 (13), 144 (100)

Table III. Mass Spectral Data for Norflurazon and Fecal Metabolites

metabolite	m/z (relative intensity)
norflurazon 1	EI, 305 (33, M^+ for ^{37}Cl), 303 (100, M^+ for ^{35}Cl), 284 (10, M − F), 145 (86), 104 (21); CI, 344 (7, M + C_3H_5), 332 (20, M + C_2H_5), 304 (100, M + H), 284 (21, M − F)
desmethyl 2	EI, 291 (33, M^+ for ^{37}Cl), 289 (96, M^+ for ^{35}Cl), 270 (12, M − F), 254 (2), 145 (100), 88 (81); CI, 330 (6, M + C_3H_5), 318 (15, M + C_2H_5), 292 (32, M + H), 290 (100, M + H), 270 (23, M − F)
methyl sulfide 6	EI, 335 (3, M^+), 290 (34, M − SCH_3), 288 (100, M − SCH_3), 261 (6), 243 (4), 204 (8), 145 (5), 88 (13); CI, 376 (1, M + C_3H_5), 364 (2, M + C_2H_5), 338 (34, M + H for ^{37}Cl), 336 (100, M + H for ^{35}Cl), 316 (20, M − F), 228 (8, M − SCH_3)
sulfoxide 9	EI, 317 (25, M^+), 302 (59, M − CH_3), 269 (100, M − SO), 255 (19), 204 (17), 145 (39); CI, 358 (5, M + C_3H_5), 346 (16, M + C_2H_5), 318 (100, M + H), 298 (26, M − F)
deschloro 10	EI, 255 (24, M^+), 236 (4, M − F), 200 (1), 172 (6), 145 (19), 125 (5), 54 (100)
sulfone 11	EI, 367 (0.2, M^+), 290 (33), 288 (100), 234 (8), 226 (4), 88 (45); CI, 408 (4, M + C_3H_5), 396 (6, M + C_2H_5), 370 (37, M + H for ^{37}Cl), 368 (100, M + H for ^{35}Cl), 348 (7, M − F), 288 (8), 254 (16)
disulfone 12	CI, 452 (2, M + C_3H_5), 440 (1, M + C_2H_5), 412 (100, M + H), 334 (35), 223 (6), 143 (100); FAB (Xe), 412 (2, M + H)

was assayed subsequently by TLC (SS 4). The resulting origin zone (R_f 0.0–0.2) was analyzed again by TLC by employing a different solvent system (SS 6). After further inspection of several of the TLC zones by LC (SS 3), two metabolites were isolated and analyzed by mass spectrometry, but plausible structures could not be assigned.

The more mobile zone (R_f >0.2) from the TLC analysis of the polar (methanol) eluent (column chromatography) was assayed by LC (SS 1), which yielded metabolites **3** and **4**. The mass spectral data used to elucidate the structures of **3** and **4** are given in Table II.

The nonpolar fraction (ethyl acetate eluent) from the column chromatography of the fecal extract was assayed by TLC (SS 4). Since the majority of the radiolabel was in one region (R_f ≈0.66), this region was assayed again by TLC using a new mobile phase (SS 5). Sulfoxide **9** (R_f 0.37) was purified by LC (SS 1) prior to spectral analysis (Table III). Although **9** could be isolated by using the new mobile phase (SS 5), the preponderance of radiocarbon remained in a different region (R_f 0.53). However, LC analysis (SS 1 and then SS 2) of this region separated norflurazon (**1**) and its metabolites (**2, 6, 10–12**), which were verified by mass spectrometry (Table III).

Tissue Analysis. Each rat was sacrificed (ether) 4 days after dosage with [^{14}C]norflurazon. All major organs and selected tissues (e.g., fat and muscle) were removed for quantification of ^{14}C by combustion in conjunction with LSC. The carcass remains were homogenized with a meat grinder (Univex), and ^{14}C was quantified by combustion of aliquots followed by LSC.

RESULTS AND DISCUSSION

The recovery of applied radiocarbon was virtually quantitative (Table IV). The fact that little (0.1% applied dose) volatile ^{14}C was produced by the rats demonstrates that the pyridazinyl moiety is not converted readily to $^{14}CO_2$ (or other volatile products) by rats.

Urine. Within 4 days after treatment with [^{14}C]norflurazon, the rats from each dose group excreted 19–28% of their applied doses in the urine. As shown in Table V, norflurazon is exhaustively and rapidly metabolized with ≤5% of the ^{14}C in the 0–1-day urine (for each dose group) present as parent compound. Six urinary metabolites of norflurazon were isolated. Except for the group 2 rats (110 mg/kg, single oral), the most abundant radiolabeled res-

Table IV. Average Distribution of Radioactivity in Female Rats Given [^{14}C]Norflurazon

	% applied dose			
	2 mg/kg[a] single, oral	110 mg/kg[a] single, oral	2 mg/kg daily[a] for 14 days, then single, oral	2 mg/kg[b] i.v.
urine				
day 0–1	18.4	8.1	24.7	24.8
day 1–4	2.9	10.4	2.3	3.6
total	21.3	18.5	27.1	28.4
feces				
day 0–1				
methanol extr	29.8	30.0	25.0	28.2
resid solids	28.5	10.1	29.7	28.8
day 1–4				
methanol extr	8.8	20.5	4.3	5.9
resid solids	10.7	18.9	6.2	7.9
total	77.8	79.5	65.3	70.7
selected tissues	0.5	0.2	0.3	0.4
carcass remains	0.4	0.4	0.5	0.5
total	100	98	93	100

[a] Average of five animals. [b] Average of two animals.

Table V. Distribution of Metabolites in the Urine (0–1 Day) of Female Rats Given [^{14}C]Norflurazon

	% ^{14}C in urine			
metabolite	2 mg/kg[a] single, oral	110 mg/kg[b] single, oral	2 mg/kg daily[a] for 14 days, then single, oral	2 mg/kg[c] i.v.
norflurazon 1	5	<1	2	<1
desmethyl 2	7	<2	<1	<2
unknown	12	14	4	6
unknown	10	22	10	10
sulfoxide 3	24	7	29	39
mercapturate 4	8	3	6	8
thiol 5	5	12	1	<1

[a] Composite of five animals. [b] Single rat. [c] Composite of two animals.

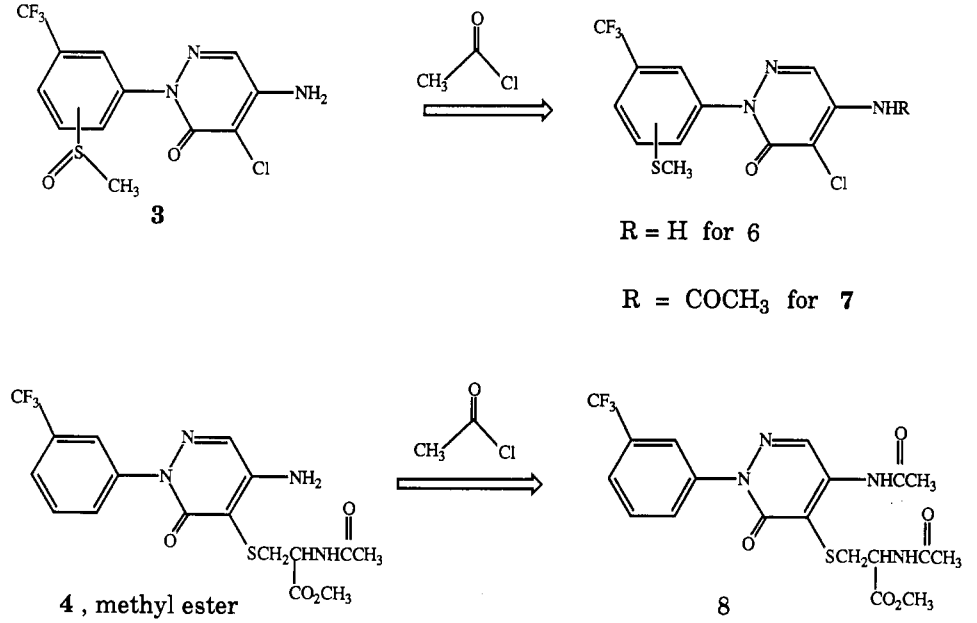

Figure 2. Acetylation of sulfoxide 3 and the methyl ester of mercapturate 4.

idue in the urine was sulfoxide **3** (24–39% of the urinary ^{14}C). Desmethylnorflurazon (**2**) accounted for 7% of the urinary radiocarbon (day 0–1) for rats given a single oral dose of [^{14}C]norflurazon at 2 mg/kg but represented ≤2% of the urinary ^{14}C (day 0–1) for the other three dose groups. Desmethylnorflurazon has been encountered previously in norflurazon studies involving soil metabolism (Eder, 1973) and rat metabolism (Karapally, 1974). The other urinary ^{14}C residues were **4** and **5**, representing up to 8 and 12% of the radiolabel in the urine, respectively. Metabolic products **3–5** have not been characterized previously as norflurazon metabolites.

Feces. For each dose group, the preponderance of the radioactivity (65–80% of the applied ^{14}C) is excreted in the feces within 4 days after treatment with [^{14}C]norflurazon. Approximately half of the radiocarbon in each fecal sample was extractable. As shown previously in the urine, norflurazon is rapidly and thoroughly metabolized; only 2–7% of the ^{14}C in the 0–1-day fecal extract remains as parent compound (Table VI). Ten metabolic products of norflurazon in the fecal extract were isolated. While no single ^{14}C residue clearly predominates, the most abundant metabolite for every dose group is sulfoxide **3** (also the most prevalent urinary metabolite). Metabolites **2**, **4**, **6**, and **9–12** each account for up to 3–7% of the ^{14}C in the extract (day 0–1). Except for desmethylnorflurazon (**2**), none of these norflurazon metabolites have been identified previously. Several miscellaneous free metabolites were present, but these each contributed <3% of the applied ^{14}C.

More than 35% of the applied dose occurred in feces as unextractable ^{14}C residues for each group of rats dosed at 2 mg/kg. We were able to solubilize some of this unextractable radioactivity by treatment with hydrolytic enzymes and even more after a harsher treatment with KOH. Although no released metabolites could be identified, no single component of the solubilized ^{14}C residue contributed more than 3% of the applied dose.

Tissues. The residue of ^{14}C in the carcass was minimal at each dose rate; less than 0.5% of the applied radiocarbon was present in the carcass remains 4 days posttreatment. Four days after dosage (all dose groups), the levels of ^{14}C in most tissues were extremely low and many were even below the limit of detection. Only the liver and kidney contained comparatively higher levels of radioactivity, and

Table VI. Distribution of Extractable Metabolites in the Feces (0–1 Day) of Female Rats Given [^{14}C]Norflurazon

	% ^{14}C in fecal extract			
metabolite	2 mg/kg[a] single, oral	110 mg/kg[b] single, oral	2 mg/kg daily[a] for 14 days, then single, oral	2 mg/kg[c] i.v.
norflurazon **1**	2	7	3	3
desmethyl **2**	3	2	3	5
sulfoxide **3**	8	10	6	11
mercapturate **4**	1	4	1	<1.0
methyl sulfide **6**	7[d]	3	1	1
unknown	2	6		
unknown	3	6		
sulfoxide **9**	3	5	4	7
deschloro **10**	...	3	2	4
sulfone **11**	5[e]	1	4	7
disulfone **12**	...	0.3	2	3

[a] Composite of five animals. [b] Single rat. [c] Composite of two animals. [d] **6** and **10** combined. [e] **11** and **12** combined.

these residues were each <0.3% of the applied radiolabel.

Conclusions. When norflurazon is given orally to rats, it is degraded extensively as evidenced by the excretion of ≤2% of the applied dose as intact norflurazon. Since there were only minor differences in excretory profiles for oral and intravenous administration routes at 2 mg/kg, it appears that norflurazon and its metabolites are absorbed readily from the gastrointestinal tract. Nine metabolites were identified in urine and feces. Eight of the metabolites have not been identified previously in rats (Karapally, 1974), but we think the discovery of these new metabolites is the result of the application of improved technology in the intervening decade. Only one identified metabolite contributed more than 10% of the applied dose and that was sulfoxide **3** (13% applied dose for combined urine and feces with intravenous group). In order to verify our structural assignment for **3**, we acetylated it with acetyl chloride. To our surprise, two derivatives formed (**6** and **7**, Figure 2; only **7** with an acetylated amino group) and both were reduced to methyl sulfides. A literature search revealed that reduction of methyl sulfoxides to methyl sulfides in the presence of acetyl chloride is a general reaction for aryl sulfoxides (Numata and Oae, 1973). The remaining identified metabolites of norflurazon each rep-

resented 1–2% of the applied dose.

Norflurazon is metabolized in rats by two major pathways: N-demethylation and probably reaction with glutathione. The central role of these pathways in the metabolism of norflurazon by rats is demonstrated by our finding that eight of the nine metabolites we identified are produced by these routes. All metabolites except **5** arose after N-demethylation. As with other aromatic xenobiotic substrates, we suspect that sulfur is added to norflurazon via initial conjugation with glutathione (Bakke, 1986). The glutathione adduct then can be converted to thiol, methyl sulfide, sulfoxide, and sulfone after β-lyase cleavage. Glutathione appears both to displace the chlorine and to attack the aromatic ring in norflurazon. Secondary glutathione metabolites (i.e., mercapturic acid **4**, thiol **5**, methyl sulfide **6**, sulfoxides **3** and **9**, and sulfones **11** and **12**) were the major metabolites of norflurazon. A minor metabolic pathway involved replacement of the chlorine in norflurazon with hydrogen.

Registry No. **1**, 27314-13-2; **2**, 23576-24-1; **3**, 121442-73-7; **4**, 121442-69-1; **5**, 121442-70-4; **6**, 121442-74-8; **9**, 121442-71-5; **10**, 121442-72-6; **11**, 121442-75-9; **12**, 121442-76-0.

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Stability of Benomyl Homologues and Their Efficacy against Sensitive and Benomyl-Resistant *Botrytis cinerea*

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Methyl, ethyl, and hexyl isocyanate homologues of benomyl (MBC-MIC, MBC-EIC, and MBC-HIC, respectively) were prepared and compared with benomyl (MBC-BIC). In water (pH 6.2), the half-lives of these compounds were 2.7–7.7 times greater at 178 μM than at 1.78 μM. They were less stable at 10 and 25 °C than at 1 °C. These compounds were tested for the protection of apples wound-inoculated with sensitive (S) and benomyl-resistant (R) isolates of *Botrytis cinerea* and stored at 1 and/or 20 °C. In the 20 and 1 °C/20 °C programs, MBC-EIC was comparable to benomyl and superior to MBC-MIC against S. Against R, MBC-EIC and MBC-MIC were cross-resistant to benomyl but much more active than benomyl. At 1 °C, MBC-MIC was negatively cross-resistant to R. MBC-HIC had slight activity to S but no activity to R.

Benzimidazole fungicides were very effective for plant protection when they were first introduced commercially in the early 1970s. Two of the widely used compounds were carbendazim, methyl 1*H*-benzimidazol-2-ylcarbamate (MBC), and its butyl isocyanate derivative benomyl, methyl [1-(butylcarbamoyl)-1*H*-benzimidazol-2-yl]carbamate (MBC-BIC). Within 2–3 years of intensive fungicide use, many fungal populations became benzimidazole-resistant and the fungicides became ineffective (Bollen and Scholten, 1971; Dekker, 1976; Delp, 1980; Elad et al., 1988; Northover, 1986; Northover and Matteoni, 1986). We found that the methyl and ethyl isocyanate homologues of benomyl (MBC-MIC and MBC-EIC, respectively) were fungitoxic to both the sensitive and the benomyl-resistant spores of *Botrytis cinerea* Pers: Nocca & Balbis (Chiba and Northover, 1988). Furthermore, MBC-EIC was effective for the protection of wounded apples inoculated with benomyl-resistant *B. cinerea* and stored briefly at 20 °C, but its efficacy under commercial cold storage was not known. Hence, the present studies were undertaken to examine the effects of temperature upon the rate of in vitro degradation of benomyl and three isocyanate homologues. The activities of these compounds for the protection of wounded apples against *B. cinerea* both sensitive and resistant to benomyl were determined under conditions that simulated commercial cold storage of apple fruit.

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MATERIALS AND METHODS

Synthesis of Compounds. Benomyl was of analytical grade (99% purity) provided by E. I. du Pont de Nemours & Co. Inc. (Wilmington, DE). The methyl, ethyl, and hexyl isocyanate homologues of benomyl (MBC-MIC, MBC-EIC, and MBC-HIC, respectively) were prepared by the reaction of MBC (99% purity) (Du Pont) with, respectively, methyl isocyanate (MIC) (Eastman Kodak Co., Rochester, NY), ethyl isocyanate (EIC) (Aldrich Chemical Co., Milwaukee, WI), and hexyl isocyanate (HIC) (Eastman Kodak Co.). The procedures were described previously (Chiba and Northover, 1988).

Identity of Compounds. The identities of benomyl and the three synthesized compounds were established by elemental analysis, which was reported earlier (Chiba and Northover, 1988), and by proton nuclear magnetic resonance (NMR), mass spectrometry (MS), and high-performance liquid chromatography (HPLC).

The NMR analyses were made with a Bruker AC-200 multinuclear FT-NMR equipped with an Aspect 3000 computer and array processor. The spectra were run in 5-mm-o.d. NMR tubes with deuterated dimethyl sulfoxide as the solvent and tetramethylsilane as the internal standard. Sample concentrations were 15–20 mg/2 mL of solvent. Spectra were collected at ambient

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