Fluopicolide: Metabolism in animals

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1 Introduction

Fluopicolide represents a new class of chemistry with a novel and unique mode of action with no cross resistance to other oomycete fungicides. It is very effective at low dose rates against a wide range of oomycete (Phycomycete) diseases including downy mildews (*Plasmopara, Pseudoperonospora, Peronospora, Bremia*), late blight (*Phytophthora*) and *Pythium* species. Crop uses include vines, potato, tomato, cucumber, melon, onion, cabbage, lettuce, leek and pepper.

The toxicokinetic behaviour of fluopicolide has been investigated in the rat, the lactating cow and the laying hen. The data from the livestock metabolism studies were performed to help assess the human dietary exposure and risk associated with the use of fluopicolide by providing data upon the nature and magnitude of residues that may be found in the meat, milk and eggs of farm animals. The rat metabolism studies were performed as a model for human metabolism, to support the toxicology database and assess the commonality of the metabolic profile in rats and crops.

Two radiolabelled forms of fluopicolide, [phenyl-U-^{14}C] and [pyridyl-2,6-^{14}C], were used (separately) in order to elucidate its metabolism in both rats and livestock (for structures see Mackenzie et al., this issue, page 209).

2 Behaviour in the rat

Studies investigating the absorption, distribution, metabolism and excretion of fluopicolide have been performed using the Sprague Dawley CD rat. The studies performed included single oral administration at the rates of 10 mg/kg (both radiolabels) and 100 mg/kg (phenyl radiolabel only) and repeated oral administrations (14 days, phenyl radiolabel only) at the rate of 10 mg/kg. Blood and plasma kinetics were investigated using a single oral dose at the nominal dose rates of 10 and 100 mg/kg body weight (both radiolabels) as were the tissue kinetics using a single oral dose at the nominal dose rates of 10 and 100 mg/kg body weight for the phenyl radiolabel and 10 mg/kg body weight for the pyridyl radiolabel. The bile kinetics were investigated using a single oral dose at the nominal dose rates of 10 and 100 mg/kg body weight for the phenyl radiolabel and at 10 mg/kg body weight for the pyridyl radiolabel.

Preliminary studies with both radiolabels indicated that the elimination of radioactivity via the expired air was less than 0.1 % of the administered dose. The recovery data following oral administration of fluopicolide are presented in Table 1. The major route of elimination was the faeces for both dose levels and both radiolabels. No sex difference was observed. There was a tendency towards a higher

Table 1: Mean recoveries of radioactivity expressed as percentage of administered dose.

Radiolabel	[U-^{14}C-phenyl]								[2,6-^{14}C-pyridyl]			
Sample	10 mg/kg				100 mg/kg				10 mg/kg			
	Males		Females		Males		Females		Males		Females	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Urine	10.0	3.7	13.1	3.3	5.4	2.4	6.6	1.0	18.8	1.5	21.4	4.6
Cage wash	1.0	0.5	2.0	0.8	1.0	0.2	1.8	1.4	2.0	1.1	5.3	1.2
Faeces	82.6	3.8	82.1	2.7	87.5	6.3	88.3	8.1	72.4	5.1	68.8	4.7
Tissues	1.3	0.1	1.0	0.1	0.8	0.2	1.0	0.2	0.7	0.4	0.5	0.0
Total	94.9	0.5	98.2	4.5	94.7	7.5	97.7	8.8	93.9	6.8	96.0	1.3

SD: Standard Deviation

urinary excretion level using the pyridyl radiolabel compared to the phenyl radiolabel which suggests that a proportion of the metabolites that were formed differed between the two radiolabels and were presumably linked to the formation of AE C657188 and AE C653711.

Following 14 daily oral administrations of [phenyl-U-^{14}C]-fluopicolide the total recovery of radioactivity was ca. 96 % with the faeces, again, being found to be the major route of elimination representing 79 % dose for the males and 73 % dose for the females. The urine was found to represent 15 % dose for the males and 22 % dose for the females. Thus it appeared that repeated dosing enhanced the elimination via the urine compared to the single oral dose. Tissue radioactivity levels were consistently low and ranged between 0.5 % dose to 1.3 % dose for the single dose studies and a mean of 0.4 % dose for the repeat dose study.

2.1 Absorption

Given the high levels of radioactivity found in the faeces, the biliary elimination of radiolabelled fluopicolide was investigated using both ring labels. Table 2 presents the percentage of the radioactivity eliminated in the first 48 hours post dose for both experiments for the dose rate of 10 mg/kg.

These data show that a large proportion of the radioactivity found in the faeces had been absorbed and then eliminated via the bile. By summing the radioactivity levels found in the urine and cage wash, tissues and bile the extent of oral absorption of [^{14}C]-fluopicolide was found to be a mean of 80.1 % dose for the phenyl radiolabel and 61.5 % dose for the pyridyl radiolabel.

Following single oral dosing using either [phenyl-U-^{14}C]-fluopicolide or [pyridyl-2,6-^{14}C]-fluopicolide the general pharma-

Table 2: Bile excretion expressed as percentage of administered dose.

Radiolabel	Males		Females	
	Mean	SD	Mean	SD
[U-^{14}C-phenyl]	70.0	6.8	73.9	8.0
[2,6-^{14}C-pyridyl]	51.6	9.3	51.7	12.8

SD: Standard Deviation

cokinetic profiles were similar between both the radiolabels and the sexes. The radiolabelled fluopicolide was absorbed moderately rapidly with mean maximal concentrations being achieved between 7 and 10 hours post dose. The only significant biological difference that was observed was between the dose levels. The difference between the high and low dose levels was that the increase in the total systemic exposure (as measured by the $AUC_{(0\text{-inf})}$) did not increase proportionately with the dose (ca. 5-6 fold against a 10-11 fold increase in dose). This was apparently due to a proportionately lower level of absorption at 100 mg/kg body weight as the mean C_{max} values were also proportionately lower. A summary of the pharmacokinetic parameters is given in Table 3.

2.2 Distribution

The highest concentrations of radioactivity in the tissues following single oral administration of [phenyl-U-^{14}C]-fluopicolide at 10 and 100 mg/kg were consistently found to be in the liver and the kidneys with the skin & fur also possessing higher concentrations in the females of the high dose group. The mean (males and females) highest concentration ob-

Table 3: Summary of the pharmacokinetic parameters following single oral administration of either [pyridyl-2,6-^{14}C]- or [phenyl-U-^{14}C]-fluopicolide at the rate of 10 and 100 mg/kg body weight.

	Sex	C_{max} (µg equiv./g)		T_{max} (hours)		$t_{0.5}$ (hours)		$AUC_{(0\text{-}168\ h)}$ (µg × h/g)		$AUC_{(0\text{-inf})}$ (µg × h/g)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Blood											
Phenyl Low dose	M	1.50	0.24	7.5	1.0	56.63	1.61	48.04	8.35	51.65	8.66
	F	1.19	0.44	5.5	2.5	120.67	26.18	52.87	8.16	73.54	12.62
Pyridyl Low dose	M	1.49	0.51	7.0	1.2	80.34	14.32	40.59	5.18	45.37	15.16
	F	1.18	0.26	6.0	1.6	140.32	25.38	45.22	4.78	67.72	12.92
Phenyl High dose	M	7.05	1.06	12.0	8.0	94.39	9.20	276.83	54.66	311.91	59.94
	F	6.22	0.57	20	8.0	124.71	28.47	325.28	42.24	466.91	117.5
Pyridyl High dose	M	6.34	1.52	8.0	0.0	79.19	8.12	217.19	28.91	248.56	38.23
	F	5.10	0.78	8.0	0.0	123.84	4.69	244.84	44.51	338.64	69.55
Plasma											
Phenyl Low dose	M	2.20	0.39	8.0	0.0	18.85	1.49	54.24	10.85	55.22	11.36
	F	1.61	0.67	6.5	3.0	19.72	6.21	38.88	9.84	40.28	8.97
Pyridyl Low dose	M	2.14	0.62	7.0	1.2	14.44	2.62	48.39	20.34	48.93	20.32
	F	1.59	0.23	6.5	1.9	12.67	2.76	30.61	5.11	30.96	5.17
Phenyl High dose	M	9.63	1.72	12.0	8.0	13.72	1.73	288.24	72.91	293.64	73.71
	F	7.03	0.32	20.0	8.0	9.52	1.24	224.08	25.98	224.68	26.04
Pyridyl High dose	M	9.18	2.60	8.0	0.0	13.48	3.31	229.20	40.10	234.51	40.13
	F	6.67	1.38	8.0	0.0	9.39	1.18	175.25	49.23	180.41	49.17

SD: Standard Deviation, M: Males, F: Females, C_{max}: maximal concentration, T_{max}: time of maximal concentration, $t_{0.5}$: terminal elimination half-life, AUC: area under curve.

served was 0.37 µg equiv./g (liver) for the 10 mg/kg group and 1.45 µg equiv./g (liver) for the 100 mg/kg group. Tissue concentrations increased, on average, by 3 to 5 times between dose groups which were not proportional to the increase in dose rate (10 times) indicating that the 100 mg/kg dose level may have passed the threshold for maximal absorption. This is supported by the data obtained in the bile excretion study and the blood/plasma kinetic study. Following single oral administration of [pyridyl-2,6-^{14}C]-fluopicolide to the male and female rat at the rate of 10 mg/kg the organs containing the highest concentrations were most consistently the liver, kidneys, spleen and the blood with the majority of tissues possessing concentrations that were below 0.1 µg equiv./g. The highest mean concentrations were observed in the kidneys for the males (0.23 µg equiv./g) and the cardiac blood for the females (0.30 µg equiv./g). No detectable radioactivity was observed in the thyroid samples from either sex. Following repeated oral administration of [phenyl-U-^{14}C]-fluopicolide to the male and female rat at the rate of 10 mg/kg the organs containing the highest concentrations were most consistently the liver (1.57 µg equiv./g), kidneys and the cardiac blood.

In the tissue kinetic study [phenyl-U-^{14}C]-fluopicolide was found to be rapidly and widely distributed into the tissues following single oral doses at the nominal rates of 10 and 100 mg/kg body weight. There was no significant sex difference. The difference in achieved concentrations in the tissues between the two dose levels was not dose proportional indicating that proportionately less of the administered radioactivity was absorbed at the higher dose rate. The highest tissue concentrations were observed in the intestine and contents in both sexes and both dose levels over several sampling times. This is probably a reflection of a combination of unabsorbed material and biliary excretion for the early samples and continued biliary excretion for later time points as was demonstrated in the bile excretion study. The next highest concentrations were consistently observed in the liver, kidneys and adrenals albeit that the concentrations were decreasing with time post dosing.

In the [pyridyl-2,6-^{14}C]-fluopicolide tissue kinetic study the radioactivity was found to be rapidly and widely distributed into the tissues following a single oral dose at the nominal rate of 10 mg/kg body weight. Thereafter a significant and rapid decrease in tissue concentrations was observed means of ca. 96 % decrease between 6/7 hours post dose and 168 hours post dose. There did not appear to be a significant sex difference. The highest radioactivity concentrations were observed in the intestine & contents at all sampling times for the males and up to 36 hours post dose for the females and probably reflect the presence of biliary excretion of radioactivity. The next highest concentrations were consistently observed in the liver, kidneys, adrenals and cardiac blood albeit with declining levels with time post dose.

2.3 Metabolism

The investigations into the metabolism of fluopicolide all demonstrated that it was capable of being extensively metabolised by the rat. The formation of the metabolites AE C657188 and AE C653711 was confirmed during the course of the bio-

transformation investigations. Generally the biotransformations observed included aromatic ring hydroxylation, hydrolysis, dealkylation, acetylation, oxidative N-dealkylation and conjugation with glucuronic acid, sulphate and glutathione. The glutathione conjugates were seen to be further metabolised by loss of glycine and glutamic acid to leave cysteine conjugates. The cysteine conjugates were seen to be further metabolised by acetylation to form the mercapturic acids or to be dealkylated and S-methylated to form S-methyl metabolites. The S-methyl metabolites were seen to be oxidised to both sulphones and sulphoxides.
Investigations into the metabolites present in the liver at 8 hours post dosing revealed that fluopicolide was already extensively metabolised with up to 13 different radioactive fractions being observed. Five of these fractions were identified as AE C653711 (0.09 % and 0.08 % dose in males and females respectively), AE 0717559 (0.02 % and 0.01 % dose), AE C643890 (0.03 % and 0.10 % dose), AE 0717560 (0.08 % and 0.09 % dose) and AE C638206 (0.04 % and 0.20 % dose in males and females respectively).
The proposed metabolic pathway in the rat is presented in Figure 1.

2.4 Elimination

Following single oral dosing using either [phenyl-U-^{14}C]-fluopicolide or [pyridyl-2,6-^{14}C]-fluopicolide the general blood plasma pharmacokinetic profiles were similar between both the radiolabels and the sexes. The radioactivity associated with fluopicolide was moderately rapidly eliminated such that the majority was eliminated by 48 hours post dose followed by a slower terminal elimination phase with a mean half-life of ca. 103 hours.
Following single oral administration of [phenyl-U-^{14}C]-fluopicolide to the male and female rat at the rates of 10 and 100 mg/kg, the major route of elimination was found to be the faeces in which between 82 % to 88 % of the radioactivity was found over the seven day sampling period. The urine was found to contain between 5 % and 13 % of the administered radioactivity over the same period. The majority of the elimination occurred over the first 48 hours for the low dose group and the first 24 hours for the high dose group.
Following single oral administration of [pyridyl-2,6-^{14}C]-fluopicolide to the male and female rat at the rate of 10 mg/kg, the major route of elimination was found to be the faeces in which 72 % (males) and 69 % (females) of the radioactivity was found over the seven day sampling period. The urine (including cage wash) was found to contain 21 % (males) and 27 % (females) of the administered radioactivity over the same period. The majority of the elimination occurred over the first 48 hours.

3 Behaviour in the laying hen

Following repeated oral administration of [^{14}C]-fluopicolide with a dose equivalent to 1 or 10 ppm in the diet for 14 days to the laying hen with either the phenyl or the pyridyl radiolabel the overall recoveries of radioactivity were quantitative and ranged from between a mean of 83 % to a mean of 96 % (see Table 4).
The majority of the administered radioactivity was recovered in the excreta (82-95 %) leaving only low levels of

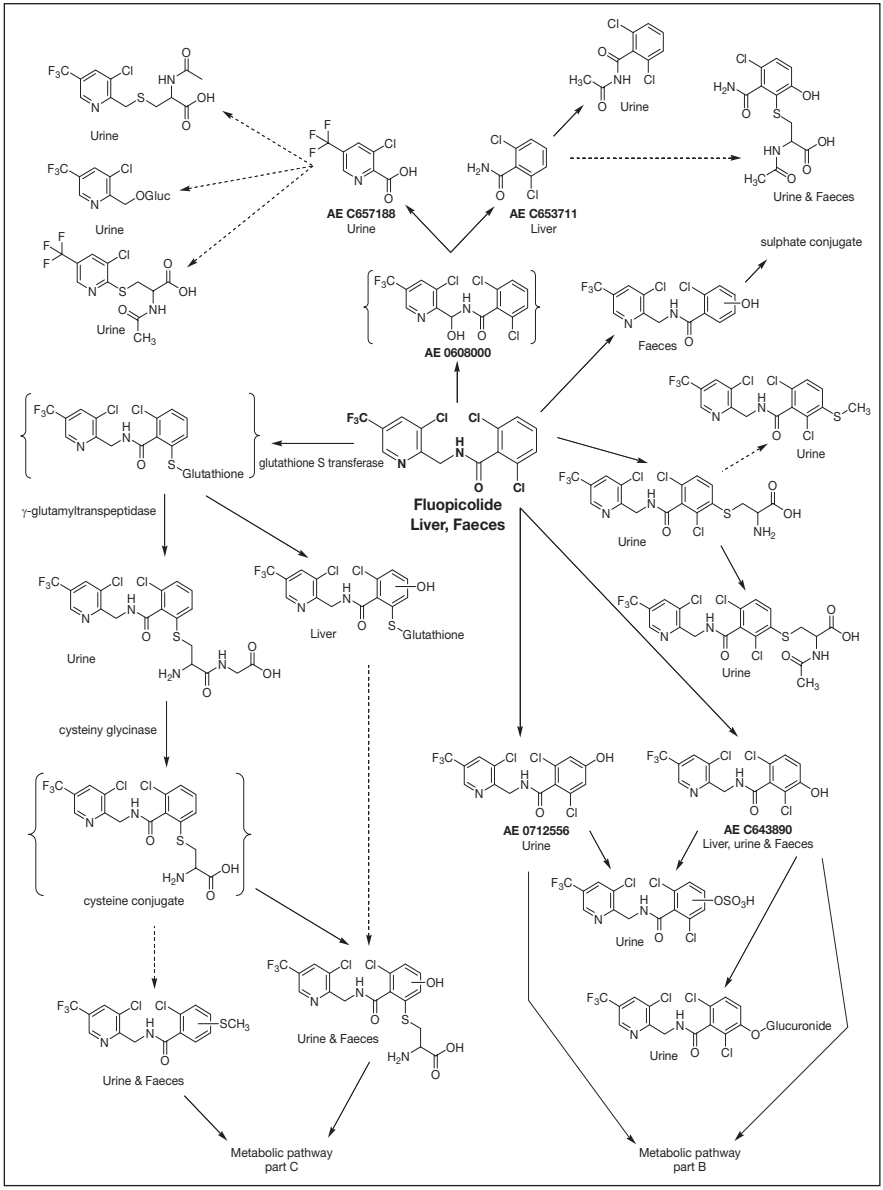

Fig. 1: A proposed metabolic pathway for fluopicolide in the rat (part A). { } = presumed intermediates.

radioactivity in the tissues and eggs. The levels of radioactivity recovered in the tissues of the phenyl radiolabel groups were consistently higher (approx. threefold) than those recovered for the pyridyl radiolabel groups. This suggests that the fate of the pyridyl radiolabel differed to some extent to that of the phenyl radiolabel which, in turn, implies that a proportion of the administered fluopicolide had been metabolised allowing the separation of the phenyl and pyridyl rings.

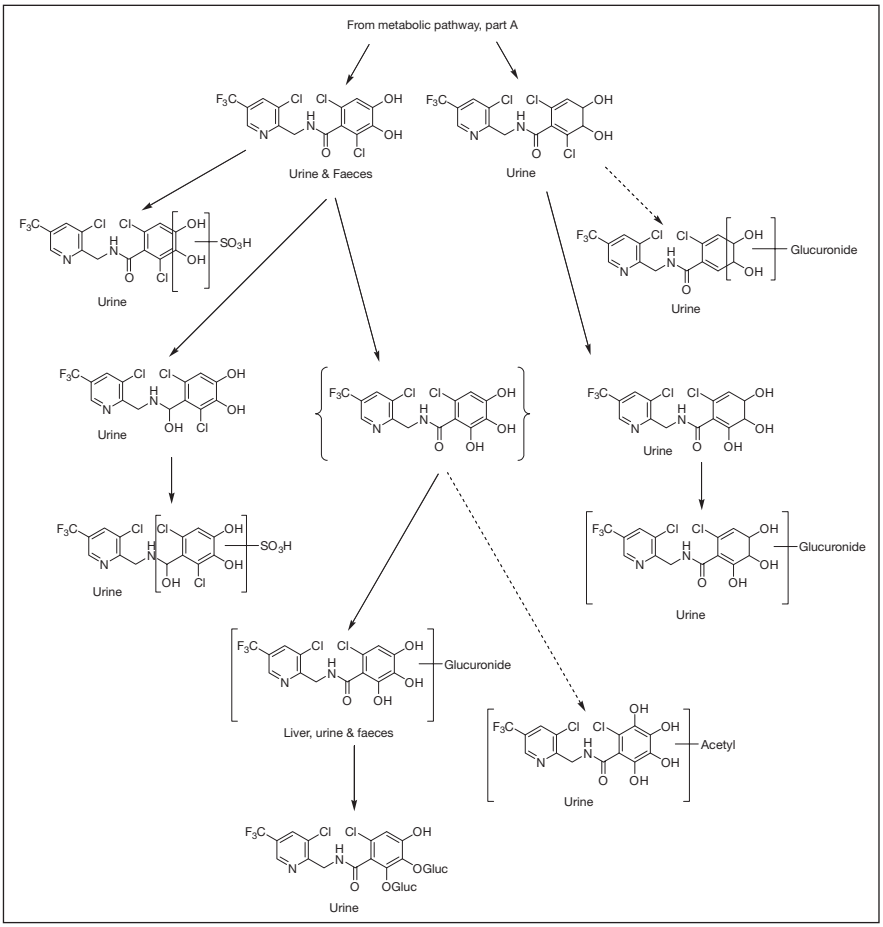

Fig. 1 (continued): A proposed metabolic pathway for fluopicolide in the rat (part B).

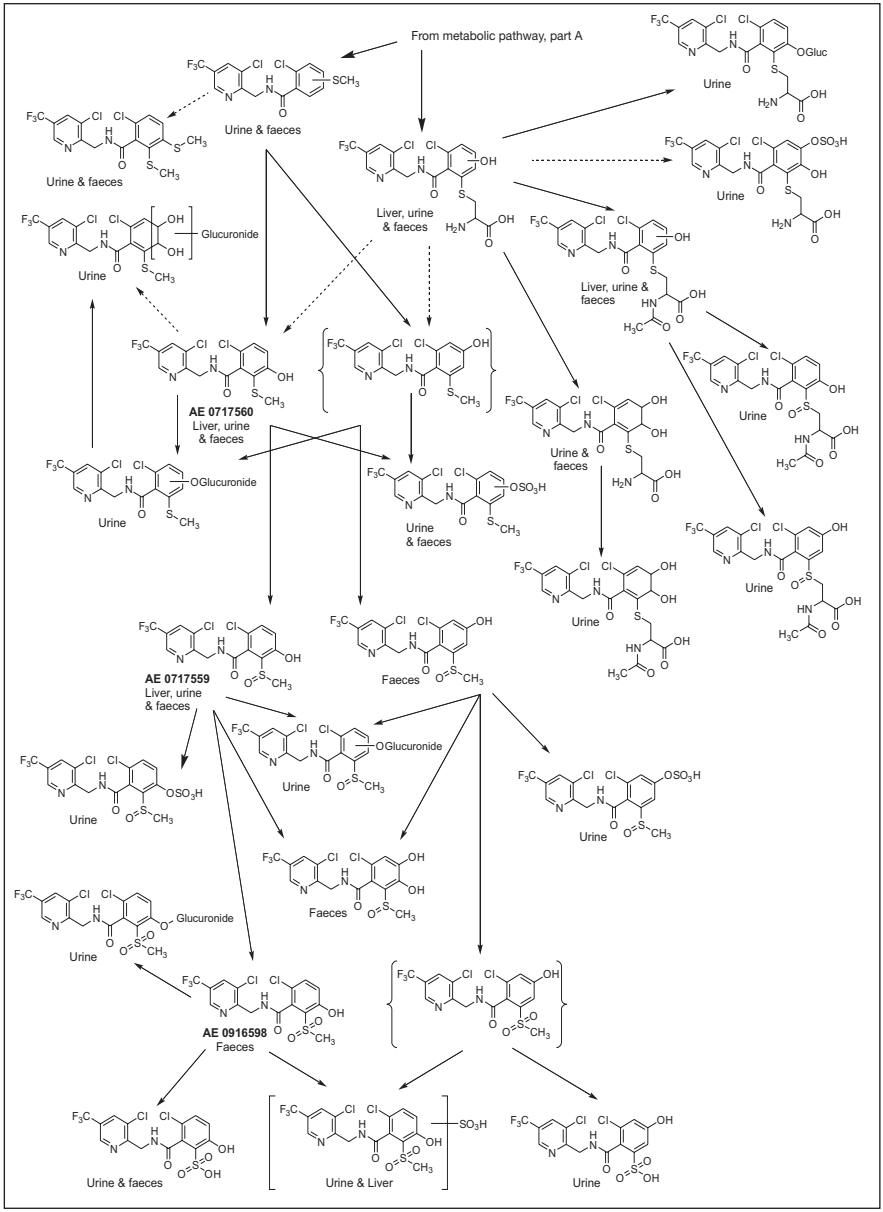

Fig. 1 (continued): A proposed metabolic pathway for fluopicolide in the rat (part C).

Table 4: Overall recovery of radioactivity from laying hens following 14 daily oral administrations of [^{14}C]-fluopicolide at the nominal dose levels of 1 and 10 ppm. Data expressed as percentage of administered dose.

Sample	[U-^{14}C-phenyl]				[2,6-^{14}C-pyridyl]			
	1 ppm diet		10 ppm diet		1 ppm diet		10 ppm diet	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Excreta	81.92	7.15	94.59	2.925	92.63	2.51	92.00	3.16
Egg white	0.04	0.03	0.04	0.02	0.04	0.01	0.02	0.01
Egg yolk	0.04	0.04	0.05	0.03	0.09	0.01	0.06	0.01
Cage wash	0.46	0.38	0.43	0.14	2.82	1.15	2.34	0.46
Final cage wash	0.28	0.23	0.21	0.06	n.a.	n.a.	n.a	n.a.
Tissues	0.24	0.07	0.23	0.06	0.09	0.02	0.06	0.01
Total	82.97	7.145	95.56	2.91	95.69	1.63	94.49	2.99

SD: Standard Deviation, n.a.: not applicable as value was included in cage wash result

At the 1 ppm dose level the mean radioactive residues found in the egg whites varied between 0.001 to 0.011 µg equiv./g with a tendency towards higher concentrations, albeit very low, for the phenyl radiolabel group. At the 10 ppm dose level this tendency was more pronounced with the concentration of radioactivity in the egg whites from the phenyl group being, on average, three times higher than those from the pyridyl dose group. In both groups the concentrations remained low, varying between 0.001 to 0.066 µg equiv./g.

At the 1 ppm dose level the mean radioactive residues found in the egg yolks varied between 0.001 to 0.023 µg equiv./g again with a tendency towards higher concentrations, albeit very low, for the phenyl radiolabel group. At the 10 ppm dose level this tendency was more pronounced with the concentration of radio-

Table 5: Concentrations of radioactivity in the tissues of laying hens following 14 daily oral administrations of [^{14}C]-fluopicolide at the nominal dose levels of 1 and 10 ppm in diet. Data expressed in terms of µg [^{14}C]-fluopicolide equivalents per gram tissue.

Sample	[U-^{14}C-phenyl]				[2,6-^{14}C-pyridyl]			
	1 ppm diet		10 ppm diet		1 ppm diet		10 ppm diet	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Liver	0.126	0.057	0.976	0.416	0.041	0.008	0.275	0.049
Skin with fat	0.008	0.003	0.069	0.011	0.003	0.001	0.022	0.011
Muscle	0.004	0.001	0.039	0.006	0.002	0.000	0.011	0.003
Fat	0.006	0.001	0.061	0.023	0.003	0.001	0.026	0.014
Blood	0.018	0.006	0.192	0.059	0.020	0.004	0.125	0.021
Plasma	0.003	0.001	0.042	0.015	0.002	0.001	0.016	0.005

activity in the egg yolks from the phenyl group being, on average, twice those from the pyridyl dose group. In both groups the concentrations remained low, varying between 0.003 to 0.198 µg equiv./g. The tissue concentrations for both radiolabels are presented in Table 5.

The highest tissue concentrations were consistently observed in the liver at both dose levels and using both radiolabels. As already discussed for the eggs and the total tissue recovery data, the radioactivity concentrations in the tissues from the dose group that received the phenyl radiolabel were higher than those from the dose group that received the pyridyl radiolabel. At the 10 ppm dose level the concentrations in the edible tissues (excluding blood and plasma) were approximately three times higher in the phenyl dose group compared to the pyridyl dose group. The blood and plasma concentrations did not consistently show the same degree of difference between the radiolabels as the edible tissues. There was no evidence of any accumulation of radioactivity in eggs or edible tissues.

The identified metabolites of fluopicolide in the hen are proposed to be formed by hydroxylation of the chlorophenyl ring in the meta and para positions to give metabolites AE 0712556 and AE C643890, respectively. Each of these metabolites is conjugated with sulphate or hydroxylated in a second position to give a proposed dihydroxy intermediate, which is further metabolised to a sulphate conjugate. Additionally a methyl sulphone conjugate of fluopicolide and AE C653711 have been observed in the liver.

In addition to the identified metabolites complementary work was performed on the unidentified polar fractions in the liver and kidney. In the phenyl radiolabel study cell fractionation demonstrated that bulk of the metabolite residues (78.2 %) were found in three cellular fractions. Water soluble low molecular weight proteins, amino acids and peptides contained 23.3 % of TRR (total radioactive residue); sulphurated glucosaminoglycans contained 29.1 % of TRR and a further 25.8 % of TRR was found in the high molecular weight proteins. There was no significant association of the radioactive residues of fluopicolide and RNA or DNA. The pyridyl radiolabel study took an alternative approach and demonstrated that the bulk of the radioactivity was associated with amino acids and peptides by use of a VYDAC HPLC column which could resolve such components. These results suggest that the residues of fluopicolide were in association with proteins in the hen liver.

The tissue distribution and egg radioactivity concentrations both indicated that higher levels were achieved with the phenyl radiolabel. The tissue recoveries demonstrated a much lower proportion of the radioactivity being left in the tissues of the pyridyl radiolabel groups compared to the phenyl radiolabel groups at the end of the study. The presence of a methyl sulphone conjugate of fluopicolide and the presence of AE C653711 in the phenyl radiolabel study both, together with the proposed metabolic pathway for fluopicolide in the rat provide a possible explanation for the observed differences in tissue and egg levels. The presence of AE C653711 shows that a proportion of the parent molecule had been cleaved, probably by oxidative N-dealkylation, to form both AE C653711 containing the phenyl portion of the parent molecule and AE C657188 which contained the

pyridyl portion of the parent. When administered to the rat [^{14}C]-AE C657188 was seen to be eliminated *via* the urine unchanged. AE C653711, on the other hand, underwent significant biotransformation which included conjugation with glutathione that was subsequently metabolised through to a mercapturic acid conjugate, a cysteine conjugate and a thiomethyl metabolite. The rat also produced such metabolites (and many more) after administration of [^{14}C]-fluopicolide. It was noteworthy that the majority of the biotransformations occurred on the

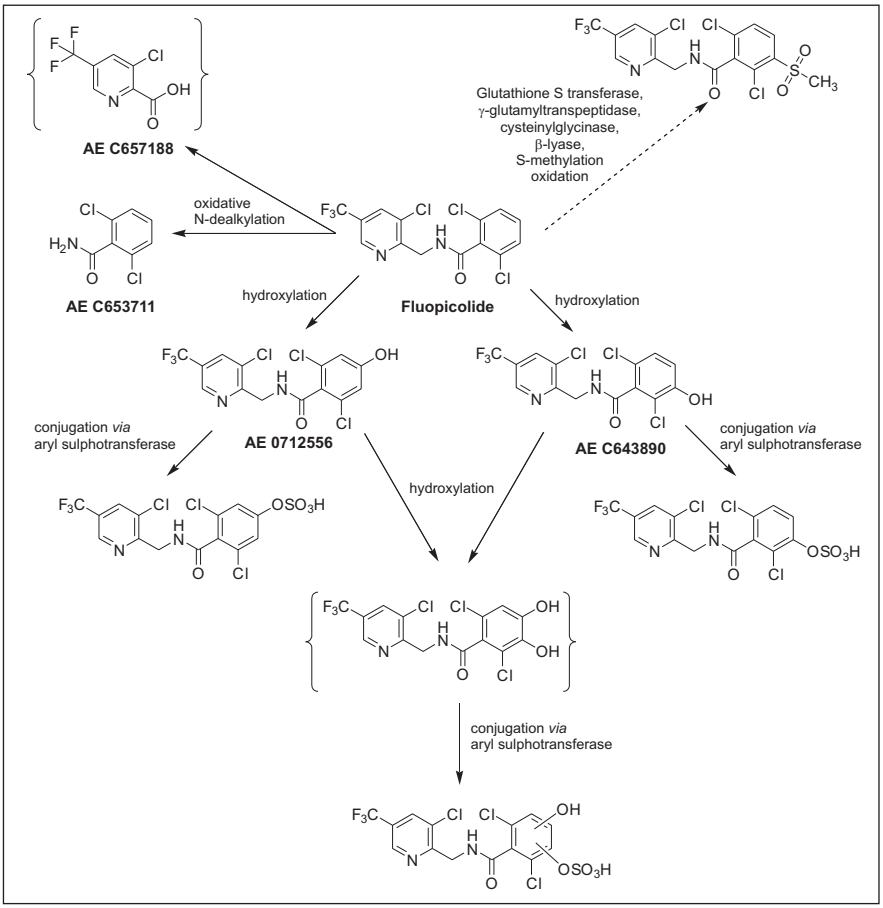

Fig. 2: The proposed metabolic pathway for [^{14}C]-fluopicolide in the laying hen. { } = presumed intermediates or metabolites.

phenyl ring of fluopicolide as opposed to the pyridyl ring. The investigations into the polar metabolites in the liver and kidney in both studies demonstrated that they were associated with amino acids, peptides and proteins. Such associations are known to occur when amino acid conjugates and thiomethyl groups are formed. Thus, assuming that the majority if not all, of these metabolites were formed on the phenyl ring (either as parent or after cleavage) then it would be expected that higher tissue levels would be observed with the phenyl radiolabel compared to the pyridyl radiolabel as more of the radioactivity would be in association with liver and kidney peptides/proteins. On the other hand in the pyridyl radiolabel experiment the radiolabelled portion of the molecule that was cleaved would be expected to be eliminated in the urine as AE C657188 resulting in relatively lower tissue radioactivity concentrations. The proposed metabolic pathway for [^{14}C]-fluopicolide in the hen following repeated administration is presented in Fig. 2.

4 Behaviour in the lactating cow

Following repeated oral administration of [^{14}C]-fluopicolide with a dose equivalent to 1 or 10 ppm in the diet for 7 days to the lactating cow with either the phenyl or the pyridyl radiolabel the overall recoveries of radioactivity were quantitative and ranged from between 76 % to a mean of 84 % (Table 6).

The majority of the administered radioactivity was recovered in the urine and faeces for both radiolabels (ca. 75 % dose for the phenyl radiolabel and 80-84 % dose for the pyridyl radiolabel) leaving only low levels of radioactivity in the tissues and milk. The levels of radioactivity recovered in the tissues of the phenyl radiolabel groups were consistently higher (approximately double) than those recovered for the pyridyl radiolabel groups (as seen for the hen). This suggests that the fate of the pyridyl radiolabel differed to some extent to that of the phenyl radiolabel which, in turn, implies that a proportion of the administered fluopicolide had been metabolised allow-

Table 6: Overall recovery of radioactivity from lactating cows following twice daily oral administrations of [^{14}C]-fluopicolide for 7 days at the nominal dose levels of 1 and 10 ppm in the diet. Data expressed as percentage of administered dose.

Sample	[U-^{14}C-phenyl]		[2,6-^{14}C-pyridyl]	
	1 ppm diet	10 ppm diet	1 ppm diet	10 ppm diet
Urine	16.81	19.34	13.50	10.71
Faeces	57.23	54.94	69.10	67.00
Cage wash	0.87	1.02	1.15	2.09
Final cage wash	0.05	0.06	n.a.	n.a.
Milk	0.14	0.13	0.09	0.08
Tissues	0.78	0.54	0.39	0.29
Total	75.88	76.03	84.23	80.17

n.a.: not applicable as value was included in cage wash result.

ing the separation of the phenyl and pyridyl rings.

At the 1 ppm dose level the radioactive residues found in the milk varied between 0.0004 to 0.002 µg equiv./g with a tendency towards higher concentrations, albeit very low, for the phenyl radiolabel group. At the 10 ppm dose level this tendency was more pronounced with the concentration of radioactivity in the milk from the phenyl group being, on average, 1.75 times higher than those from the pyridyl dose group. In both groups the concentrations remained low, varying between 0.007 to 0.019 µg equiv./g. The tissue concentrations for both radiolabels are presented in Table 7.

The highest tissue concentrations were consistently observed in the liver at both dose levels and using both radiolabels.

Comparison of the concentrations observed in the tissues in the 1 ppm experiments shows that equivalent concentrations were obtained in all the tissues except the liver and muscle. In the case of the muscle the levels in the phenyl radiolabel experiment were below the level of quantification whilst those obtained in the pyridyl dose group were very low (0.001 µg equiv./g) but measurable. For the liver however the concentration observed in the phenyl radiolabel experiment was ca. 16 times that observed in the pyridyl experiment.

Comparison of the concentrations observed in the tissues in the 10 ppm experiments shows that equivalent concentrations were obtained in the fat blood and plasma. The concentrations observed in the muscle and kidney samples from the phenyl radiolabel experiment were approximately twice those observed for the pyridyl experiment. As seen in the 1 ppm data, the concentration of radioactivity in the liver observed in the phenyl radiolabel experiment was ca. 14 times that observed in the pyridyl experiment.

The identified metabolites of fluopicolide in the cow are proposed to have been formed by hydroxylation of the chlorophenyl ring in the meta and para positions to give metabolites AE 0712556 and AE C643890 respectively. Each of these metabolites is conjugated with sul-

Table 7: Concentrations of radioactivity in the tissues of lactating cows following twice daily oral administrations of [^{14}C]-fluopicolide for 7 days at the nominal dose levels of 1 and 10 ppm in the diet. Data expressed in terms of µg [^{14}C]-fluopicolide equivalents per gram tissue.

Sample	[U-^{14}C-phenyl]		[2,6-^{14}C-pyridyl]	
	1 ppm diet	10 ppm diet	1 ppm diet	10 ppm diet
Liver	0.900	0.644	0.058	0.449
Kidney	0.026	0.302	0.033	0.196
Fat	0.006	0.040	0.004	0.041
Muscle	< LOQ	0.024	0.001	0.012
Blood	0.011	0.088	0.010	0.074
Plasma	0.013	0.100	0.011	0.082

LOQ: Limit of quantification

phate or glucuronic acid. Alternatively metabolites AE 0712556 or AE C643890 were hydroxylated in a second position to give a proposed dihydroxy intermediate, which is further metabolised to give either a sulphate or glucuronide conjugate. The presence of polar components in the enzyme hydrolysate of liver and kidney that were associated with peptides was also demonstrated by HPLC.

In addition to the identified metabolites complementary work was performed on the unidentified polar fractions in the liver and kidney. Fractionation of the liver and kidney cells showed that the majority of the radioactivity was associated with proteins and amino acids (63 % in the liver, 69 % in the kidney). It is possible that fluopicolide was being metabolised via conjugation with glutathione. The glutathione metabolites produced could then undergo cleavage by β-lyase leaving metabolites that could bind to proteins and amino acids. There was a lack of sig-

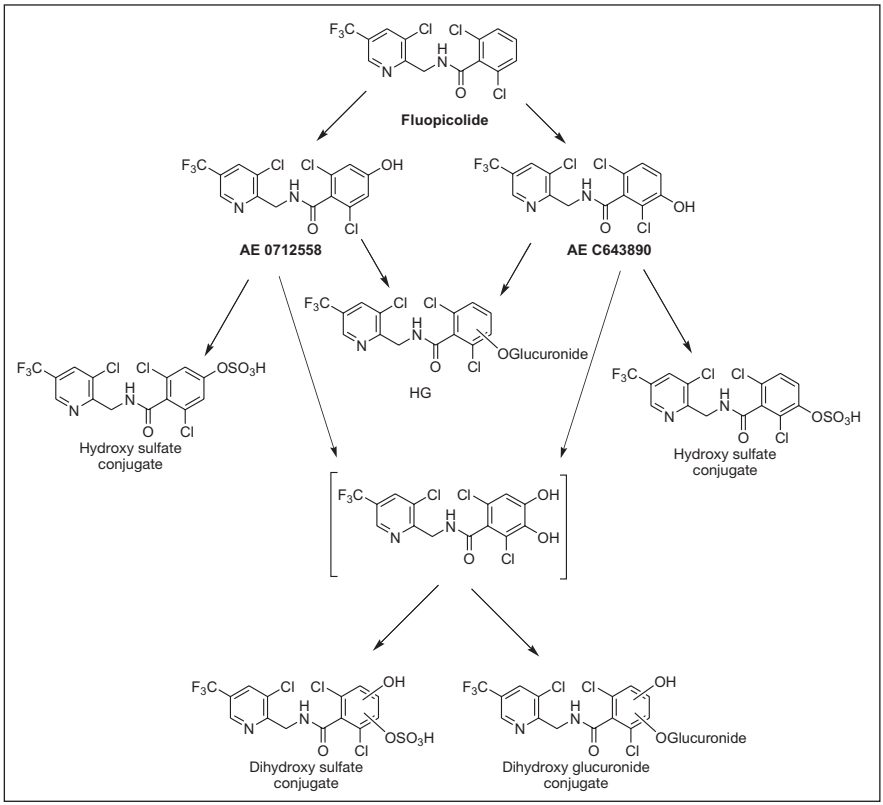

Fig. 3: The proposed metabolic pathway for [^{14}C]-fluopicolide in the lactating cow.

nificant incorporation of any protein-bound residues into RNA or DNA suggesting that the metabolic pathway was acting as a de-activation mechanism in this case. The pyridyl radiolabel study took an alternative but complementary approach and demonstrated that the bulk of the radioactivity in the unidentified polar fractions was associated with amino acids and peptides by use of a VYDAC HPLC column which resolved the components into 14 separate fractions for the liver and to below the LOQ for the kidney.

As was observed in the poultry metabolism studies, there was a tendency for higher radioactivity concentrations to be found in the phenyl radiolabel experiment compared to the pyridyl radiolabel experiment. Given that both radiolabels produced the same hydroxylated metabolites and conjugates thereof, it would appear that the difference must be due to differing fates of the phenyl and pyridyl groups following a cleavage of the parent molecule. In both the hen and the cow the highest concentrations of radioactivity were observed in the liver and the livers of both species were found to contain polar fractions that were very difficult to extract and identify but were demonstrated to be associated with some of the liver and kidney proteins. In the case of the hen (and indeed the rat) cleavage products were observed as were metabolites that would have been produced following initial conjugation with glutathione. In the case of the cow the evidence is more circumstantial as the presence of cleavage products and thiomethyl metabolites could not be confirmed. It is assumed however that they had been formed and that the explanation that has already been given for the hen in this chapter offers the best hypothesis for explaining both the difference in tissue/milk concentrations between the radiolabels and the presence of radioactivity associated with proteins.

The proposed metabolic pathway for [^{14}C]-fluopicolide in the lactating cow following repeated administration is presented in Fig. 3.

5 Summary

Metabolism of fluopicolide in animals

The metabolism of fluopicolide has been studied in the rat, cow and hen.

In the rat following oral dosing with [^{14}C]-fluopicolide at a nominal dose of 10 mg/kg the absorption was found to be moderately rapid with maximal blood concentration being achieved between 7 and 10 hours. The extent of absorption was found to be between 61.5 % to 80.1 % for both sexes. Tissue radioactivity levels were consistently low. The major route of elimination was the faeces at ca. 80 % dose. The investigations into the metabolism of fluopicolide all demonstrated that it was capable of being extensively metabolised by the rat. The biotransformations observed included aromatic ring hydroxylation, hydrolysis, acetylation, oxidative N-dealkylation and conjugation with glucuronic acid, sulphate and glutathione.

Fluopicolide, when orally administered to lactating cows or laying hens, following a repeat dose regime, is absorbed but then is rapidly and extensively eliminated (75 % to 95 %) with minimal transfer to tissues, milk and eggs. The profile of metabolism of fluopicolide in the hen and the cow was similar to that of the rat and involved the hydroxylation of the chlorophenyl ring in the *meta* and *para*

positions followed by further hydroxylation to form di-hydroxy products and/or conjugation with sulphate (cow and hen) or glucuronic acid (cow) conjugate. Additionally a methyl sulphone conjugate of fluopicolide and AE C653711 were observed in hen liver.

Zusammenfassung

Metabolismus von Fluopicolide in Tieren

Der Metabolismus von Fluopicolide wurde an Ratten, Kühen und Hennen untersucht.
Bei Untersuchungen wurde nach einer oralen Verabreichung von [^{14}C]-Fluopicolide mit einer Dosis von 10 mg/kg eine moderate Absorptionsgeschwindigkeit festgestellt. Die höchsten Konzentrationswerte im Blut traten nach 7-10 Stunden auf. Die Absorptionsrate lag bei beiden Geschlechtern zwischen 61,5 % und 80,1 %. Die im Gewebe gemessene Radioaktivität fiel durchgängig sehr gering aus. Als Hauptausscheidungsweg wurde die Fäzes mit einem Anteil von ca. 80 % ermittelt. Die Untersuchungen des Abbaus von Fluopicolide zeigten insgesamt, dass der Wirkstoff von Ratten ausgiebig metabolisiert wird. Die beobachteten Umwandlungen umfassten die Hydroxylierung aromatischer Ringverbindungen, Hydrolyse, Acetylierung, oxidative N-Dealkylierung sowie Konjugation mit Glucuronsäure, Sulfat und Glutathion.
Wiederholte orale Verabreichung von Fluopicolide an laktierende Kühe oder an Legehennen wurden zunächst absorbiert, dann aber sehr schnell und umfassend ausgeschieden (75 % bis 95 %), bei minimalem Nachweis in Gewebe, Milch bzw. Eiern. Der Metabolismus von Fluopicolide in Legehennen und Kühen war vergleichbar zu den Befunden bei Ratten. Es kam aber auch zur Hydroxylierung des Chlorophenolringes in der *meta*- und *para*-Position, gefolgt von einer weiteren Hydroxylierung, die die Bildung von Dihydroxyverbindungen und/oder Sulfatkonjugaten (in Kuh und Henne) sowie Glucuronsäureverbindungen (in der Kuh) zur Folge hatte. Zusätzlich wurde in der Leber von Legehennen noch ein Methylsulfonkonjugat von Fluopicolide und AE C653711 festgestellt.

Résumé

Le fluopicolide: le métabolisme chez les animaux

Le métabolisme du fluopicolide a été étudié chez le rat, la vache et la poule.
Après l'administration chez le rat d'une dose nominale orale de fluopicolide marqué au carbone 14 de 10 mg/kg, on a observé une absorption modérément rapide, la concentration sanguine maximale étant atteinte en 7 à 10 heures. Le degré d'absorption était compris entre 61,5 % et 80,1 % chez les deux sexes. Le taux de radioactivité tissulaire est resté constamment faible. La principale voie d'élimination est la voie fécale, avec environ 80 % de la dose administrée. Les analyses métaboliques du fluopicolide ont toutes montré qu'il est en mesure d'être extensivement métabolisé par le rat. Les biotransformations observées incluaient l'hydroxylation du noyau aromatique, l'hydrolyse, la N-désalkylation oxydative et la conjugaison avec l'acide glucuronique, le sulfate et le glutathione.
Lorsqu'il est administré par voie orale à des vaches allaitantes ou à des poules pondeuses suivant un schéma à doses itératives, le fluopicolide est absorbé puis éliminé rapidement et extensivement

(75 % à 95 %) et son passage dans les tissus, le lait et les oufs est minime. Le profil métabolique du fluopicolide chez la poule et la vache était analogue à celui observé chez le rat; il impliquait l'hydroxylation de l'anneau chlorophényl aux positions *méta* et *para*, suivie d'une hydroxylation plus poussée pour former les produits di-hydroxy et/ou une conjugaison au sulfate (chez la vache et la poule) ou à un conjugué de l'acide glucuronique (chez la vache). En outre, on a retrouvé le conjugué méthyle sulfonique du fluopicolide et l'AE C653711 dans le foie de la poule.

Resumen

Fluopicolide: Metabolismo en animales

El metabolismo de fluopicolide fué estudiado en la rata, vaca y gallina.
En la rata, después de administrar oralmente [^{14}C]-fluopicolide a una dosis nominal de 10 mg/kg, se encontró que la absorción fué moderadamente rápida, con una concentración máxima en la sangre después de 7 a 10 horas. El grado de absorción encontrado fué de 61,5 % a 80,1 % para ambos sexos. Los niveles de radioactividad en el tejido fueron consistentemente bajos. La mayor ruta de eliminación fué por vía fecal, con aprox. 80 % de la dosis. Todas las investigaciones sobre el metabolismo de fluopicolide demostraron que es capaz de ser extensivamente metabolizado por la rata. Las biotransformaciones observadas incluyeron hidroxilación del anillo aromático, hidrólisis, acetilación, N-dealkilación oxidativa y conjugación con ácido glucurónico, sulfato y glutationa.
Fluopicolide, al ser administrado oralmente a vacas lactantes o a gallinas ponedoras, siguiendo un régimen de dosis repetidas, es absorbido pero rápidamente y extensivamente eliminado (75 % a 95 %) con mínima translocación a tejidos, leche y huevos. El perfil del metabolismo de fluopicolide en la gallina y en la vaca fué similar al de la rata e involucró la hidroxilación del anillo clorofenilo en las posiciones *meta* y *para* seguido por una hidroxilación adicional para formar productos di-hidroxi y/o conjugación con sulfato (vaca y gallina) o ácido glucurónico (vaca) conjugado. Adicionalmente se observó un conjugado metil sulfónico de fluopicolide y AE C653711 en el hígado de gallina.

Резюме

Метаболизм флуопиколида в животных

Метаболизм флуопиколида исследовался на крысах, коровах и курах.
В исследованиях после оральной дачи меченного C14 флуопиколида в количестве 10 мг/кг наблюдалась умеренная скорость поглощения. Наиболее высокие значения концентрации в крови отмечались спустя 7-10 часов. У особ обоих полов степень поглощения лежала в пределах от 61,5 % до 80,1 %. Измеренная в тканях радиоактивность во всех случаях была очень низкой. Основной путь выведения – с экскрементами, на который приходится ок. 80 %. В общем итоге исследования по разложению флуопиколида показали, что крысами действующее вещество сильно метаболизируется. Наблюдались следующие процессы превращения: гидроксилирование ароматических кольцевых соединений, гидролиз, ацетилирование, окислительное N-деалкили-

рование, а также конъюгация с глюкуроновой кислотой, сульфатом и глутатионом.

При повторной оральной даче флуопиколида лактирующим коровам или курицам-несушкам вещество сначала абсорбировалось, но затем очень быстро и полно выделялось (от 75 % до 95 %) при минимальных остаточных концентрациях в тканях, молоке или яйцах. Метаболизм флуопиколида в курицах и коровах был сопоставим с результатами у крыс, однако, имело место также гидроксилирование хлорофенолового кольца в положениях мета и пара, за которым последовало следующее гидроксилирование с образованием дигидроксисоединений и/или сульфатных конъюгатов (у коровы и курицы) и соединений глюкуроновой кислоты (у коровы). В печени куриц дополнительно обнаружен метилсульфоновый конъюгат флуопиколида и AE C653711.

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