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NATURE AND ROLE OF XENOBIOTIC METABOLIZING ESTERASES IN RAT LIVER, LUNG, SKIN AND BLOOD

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Abstract—In the present study, the distribution and nature of esterases in the rat which hydrolysed fluazifop-butyl, carbaryl, paraoxon and phenylacetate were investigated. V_{max} and K_m values for the hydrolysis reactions were determined. Fluazifop-butyl was hydrolysed to fluazifop by rat liver (V_{max} μmol/min/g microsomes 6.2 ± 0.4; cytosol 6.84 ± 0.85), lung (V_{max} microsomes 0.38 ± 0.1; cytosol 1.5 ± 0.32) and skin (V_{max} microsomes 0.02 ± 0.0015; cytosol 0.4 ± 0.06) and by plasma (V_{max} μmol/min/mL 5.8 ± 0.48) and red blood cells (V_{max} 0.03 ± 0.015). Significant inhibition by paraoxon and bismitrophenol phosphate indicated the involvement of carboxylesterases. Carbaryl was hydrolysed by liver, lung and skin at a lower rate by microsomal fractions (V_{max} nmol/min/g 2.1 ± 0.25, 1.6 ± 0.25, 0.2 ± 0.035, respectively) compared to cytosolic fractions (V_{max} 6.7 ± 0.75, 1.4 ± 0.36, 0.5 ± 0.12) and plasma (V_{max} nmol/min/mL 3.0 ± 0.25). Hydrolysis involved carboxylesterases. Paraoxon was hydrolysed by paraoxonases/arylesterases only in the plasma (V_{max} nmol/min/mL 246 ± 12) and microsomal fractions from liver (V_{max} 330 nmol/min/g ± 25) and lung (V_{max} 2 ± 0.25). Phenylacetate was hydrolysed by both microsomal and cytosolic fractions from all tissues studied. Hydrolysis involved arylesterases in the microsomes and carboxylesterases in the cytosol. Extrahepatic hydrolysis may be important following some routes of exposure to xenobiotic esters.

Hydrolysis by esterases is a major route for the detoxification of xenobiotic esters in humans. Esterases were originally classified by Aldridge [1] into three groups from their interaction with organophosphates: 'A' esterases which hydrolyse organophosphates, 'B' esterases which are inhibited by organophosphates and include cholinesterase (EC 3.1.1.8) and carboxylesterase (EC 3.1.1.1) and 'C' esterases which do not interact. A esterases include paraoxonase now classifed as phosphoric triester hydrolase (EC 3.1.8.1) as a separate enzyme from arylesterase (EC 3.1.1.2) [2].

Humans are exposed to a range of xenobiotic esters used as pesticides both occupationally and in the general environment. Exposure to pesticides may occur by inhalation, absorption through the skin or by ingestion, although dermal absorption is thought to be the major route. First pass metabolism, in these extrahepatic tissues following absorption, may influence the toxic effects of the absorbed pesticides.

The aim of this study was to determine the distribution of the esterases hydrolysing three ester pesticides, fluazifop-butyl, carbaryl and paraoxon (the active P-oxon of parathion), in the liver, lung, skin and blood of the rat. Distribution of phenylacetate esterase was also determined.

Fluazifop-butyl [butyl-2-(4-(5-trifluoromethyl-2-pyridinyl)oxy)phenoxy propanoate], is an alkoxy-phenoxy propionate herbicide which may be dermally absorbed by humans from spray formulations. Fluazifop-butyl is hydrolysed rapidly to fluazifop acid, the major metabolite, which is excreted in the urine. No unchanged fluazifop-butyl is seen in the blood after oral or dermal dosing to human volunteers [3]. Carbaryl (1-naphthyl methylcarbamate) is a carbamate anti-cholinesterase insecticide, which reversibly inhibits acetylcholinesterase. The major degradative pathway of carbaryl in the liver involves hydroxylation followed by conjugation [4]. Carbaryl is hydrolysed to 1-naphthol by carboxylesterase and then conjugated to naphthol glucuronide and sulphate [5, 6]. Paraoxon is the active oxon of the organophosphate pesticide parathion [7]. Parathion is activated by microsomal enzymes to paraoxon. Paraoxon [*o,o*-diethyl-*o*-(4-nitrophenyl)phosphate] is an irreversible inhibitor of acetylcholinesterase and is hydrolysed by paraoxonoase to diethylphosphoric acid and *p*-nitrophenol.

Inhibitory studies were carried out to distinguish the esterases involved in hydrolysis of the three substrates. The classical inhibitors paraoxon, BNPP (bis-nitrophenol phosphate), mercuric chloride and physostigmine were used.

METHODS AND MATERIALS

Chemicals

Fluazifop-butyl, fluazifop and carbaryl were gifts from the Central Toxicology Laboratory, I.C.I. plc (Macclesfield, Cheshire). All other chemicals were obtained from the Sigma Chemical Co. (Poole, U.K.).

Animals

Adult male Wistar rats, weighing 180 g were obtained from the Comparative Biology Centre at the University.

Preparation of subcellular fractions

Rats were killed by cervical dislocation. Liver,

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lung and dorsal skin were removed. Liver and lung tissues were washed in ice-cold 0.34 M sucrose buffer, pH 7.0. The whole of the dorsal area of skin was shaved using electric clippers (Ostler) before removal of the skin. The skin was placed in a Petri dish on ice and the subcutaneous fat and vascular tissue were removed from the dermis.

Portions (1 g) of each of the tissues were weighed and finely minced in 10 mL 0.34 M sucrose buffer, pH 7.0. Tissue homogenates were prepared with a cooled polytron homogenizer (Kinematic) using two bursts of 10 sec duration at full power, with cooling between the bursts for skin (dermis and epidermis) and two bursts at 2 sec duration at full power for liver and lung. Tissue homogenates were centrifuged in a Sorvall RC 5B refrigerated centrifuge at 1800 *g* for 5 min at 4° to remove tissue debris. The supernatant was further centrifuged at 10,000 *g* for 10 min at 4° to remove mitochondria and nuclei. The post-mitochondrial fraction was then centrifuged in a Sorvall ultra-centrifuge at 100,000 *g* for 1 hr and 10 min. The supernatant (cytosol) was removed and retained and the microsomes were resuspended by homogenizing in a volume of 10 mL 50 mM Tris–HCl buffer pH 7.5 using a glass to glass homogenizer. Washed microsomes were isolated by recentrifuging at 100,000 *g* for 1 hr 10 min and were resuspended into 1 mL 50 mM Tris–HCl pH 7.5 for lung and skin and 5 mL for liver. The microsomal and cytosolic fractions were stored in aliquots at −80° before analysis. Loss of active protein with the nuclear and mitochondrial fractions was similar for liver, lung and skin and accounted for 10% activity. Protein recovery was determined by the method of Peterson [8]. Microsomal protein recoveries were 27.8 ± 2.2, 8.65 ± 1.5 and 2.2 ± 0.15 mg/g and cytosol 32.5 ± 1.0, 49.2 ± 4.0 and 8.8 ± 1.2 mg/g (mean ± SEM) for liver, lung and skin, respectively. However, metabolic activities were expressed relative to the wet weight of the tissue and were not corrected for recovery.

Blood (10 mL) was removed from anaesthetized rats by cardiac puncture and placed in heparinized tubes. Plasma and red blood cells were separated by centrifugation using a Mistral 3000 centrifuge, at 880 *g* for 5 min. The red blood cells were washed using an equal volume of isotonic saline. After centrifugation at 880 *g* for 10 min the upper layer (white cells) was removed and discarded. This procedure was again repeated. The red blood cells were then stored at −80° until required for analysis. Thawing lysed the red cells.

Incubations

Fluazifop-butyl. An aliquot of the microsomal or cytosolic fraction equivalent to 0.4, 5 or 30 mg original wet weight liver, lung or skin, respectively, (or 10 μL plasma or 50 μL lysed red blood cells) was incubated with fluazifop-butyl (final concentration of 0.02–1 mM). Incubations were carried out in a final volume of 500 μL in 50 mM Tris buffer pH 8.0, containing 0.1 mM calcium chloride at 37°. Reactions were started by the addition of fluazifop-butyl (5–20 μL 1.0 and 10 mM stock, in acetonitrile) and stopped after 4 min for liver and plasma and 10 min for lung, skin and red blood cells by the addition of an equal volume of 6% perchloric acid, containing 10 μg/mL *p*-toluic acid (internal standard). Tubes were vortexed and centrifuged at 5440 *g* for 5 min to remove protein. Supernatant (80 μL) was injected onto reverse phase HPLC as described. Control incubations for estimation of spontaneous hydrolysis of fluazifop-butyl were conducted with buffer alone.

Carbaryl. Carbaryl in 15 μL methanol to give a final concentration of 0–50 μM per incubation was added to glass conical bottomed tubes and the methanol was evaporated under nitrogen. Incubations were carried out in a final volume of 1 mL in 0.1 M phosphate buffer pH 7.25 at 37°. Reactions were started by addition of an aliquot of microsomal or cytosolic fraction equivalent to 5, 10 and 30 mg of liver, lung or skin, respectively, or 20 μL of plasma and 50 μL of lysed red blood cells. Samples were then vortexed for 5 sec. Reactions were stopped after 20 min by protein precipitation following addition of ethanol (500 μL per incubation) and centrifugation at 5400 *g* for 5 min. Supernatant (50 μL) was injected onto reverse phase HPLC as described. Control incubations for spontaneous hydrolysis of carbaryl containing 0.1 M phosphate buffer alone were conducted in parallel.

Paraoxon. An aliquot of the microsomal or cytosolic fraction equivalent to 0.5, 20 or 30 mg liver, lung or skin, respectively, or 10 μL plasma or 50 μL lysed red blood cells was incubated with paraoxon (final concentration of 0.1–1 mM). Incubations were carried out in a final volume of 500 μL in 50 mM Tris buffer pH 8.0, containing 0.1 mM calcium chloride at 37°. Reactions were started by the addition of paraoxon (5–50 μL of a 10 mM stock, freshly prepared in Tris buffer) and stopped after 15 min by the addition of an equal volume of 6% perchloric acid, containing 10 μg/mL *p*-toluic acid. Tubes were vortexed and centrifuged at 5440 *g* for 5 min. Supernatant (80 mL) was injected onto reverse phase HPLC as described. Control incubations containing 50 mM Tris buffer pH 8.0 but not tissue fraction were conducted in parallel.

Phenylacetate. Microsomal and cytosolic protein equivalent to 2, 20 or 30 mg liver, lung and skin, respectively, or 10 μL plasma or 50 μL lysed red blood cells were incubated with a final concentration of 0.5–4 mM phenylacetate. Incubations were carried out in a final volume of 3 mL in 50 mM phenylacetate. Incubations were carried out in a final volume of 3 mL in 50 mM Tris buffer pH 8.0, containing 0.1 mM calcium chloride at 37°, in a cuvette. Reactions were started with the addition of phenylacetate (5–20 μL of 600 mM phenylacetate stock in dimethyl sulphoxide). Formation of phenol with time was monitored at 270 nm in a spectrophotometer. Control incubations containing 50 mM Tris buffer pH 8.0 alone were conducted in parallel.

Inhibition studies

Microsomal or cytosolic protein equivalent to 0.4, 5 or 30 mg of liver, lung or skin, respectively, or 10 μL of plasma was preincubated at 37° with 0.1 mM paraoxon, 0.1 mM BNPP, 0.1 mM physostigmine or 0.1 mM mercuric chloride for 5 min prior to the addition of fluazifop-butyl. Final concentrations of

fluazifop-butyl were 300 μM for liver, 50 μM for lung, 30 μM for skin and 200 μM for plasma. Control incubations without any inhibitors in the incubation were carried out in parallel. Inhibition was estimated by expressing remaining activity in the presence of the inhibitor as a percentage of the control activity.

Similarly, microsomal or cytosolic protein equivalent to 5, 10 and 30 mg liver, lung and skin, respectively, or 20 μL of plasma was preincubated with 0.1 mM BNPP or 0.1 mM physostigmine for 5 min before carbaryl (25 μM) was added. Control incubations with no inhibitor were carried out in parallel.

Microsomal and cytosolic protein equivalent to 2, 20 or 30 mg liver, lung or skin, respectively, or 10 μL plasma was preincubated with 0.1 mM paraoxon, 0.1 mM mercuric chloride, 0.1 mM BNPP or 0.1 mM physostigmine for 5 min before phenylacetate (2 mM) was added. Control incubations with no inhibitor were carried out in parallel.

HPLC assays

Fluazifop-butyl. A Kontron system with dual wavelength UV detection fitted with a Spherisorb ODS (5 μ, 15 cm) C18 reverse phase HPLC column was used with a mobile phase of orthophosphoric acid (0.072%, w/v)/acetonitrile (40:60) at a flow rate of 1 mL/min. Fluazifop-butyl and fluazifop acid were monitored at 270 nm and *p*-toluic acid at 250 nm. The retention times were internal standard 2.5 min, fluazifop 3.5 min and fluazifop-butyl 12.5 min. Calibration curves for fluazifop acid were established. Fluazifop acid standards (0–120 μM final concentration in 50 mM Tris buffer pH 8.0) mixed with an equal volume of 6% perchloric acid, containing 10 μg/mL *p*-toluic acid were injected onto the column (80 μL) without neutralization.

Carbaryl. A Spherisorb ODS (5 μ, 15 cm) C18 reverse phase HPLC column was used with a mobile phase of water/acetonitrile (60:40) at a flow rate of 1.4 mL/min, pumped by a Waters 510 HPLC pump. 1-Naphthol was measured in the eluent by using a Perkin–Elmer 3000 fluorimeter set at excitation wavelength 296 nm and emission wavelength 460 nm. The retention time for 1-naphthol was 6.2 min. A calibration curve for 1-naphthol was established: 0–2 nmol 1-naphthol in 1 mL 0.1 M phosphate buffer pH 7.25 was added to 500 μL of absolute ethanol. Aliquots (50 μL) were injected onto the column.

Paraoxon. A Kontron HPLC system with dual wavelength UV detection fitted with a Spherisorb ODS (5 μ, 15 cm) C18 reverse phase column was used with a mobile phase of orthophosphoric acid (0.072%, w/v)/methanol (45:55) at a flow rate of 1 mL/min. Paraoxon and *p*-nitrophenol and *p*-toluic acid were monitored at 250 nm. The retention times were *p*-nitrophenol 4.5 min, *p*-toluic acid 6.4 min and paraoxon 10 min. A calibration curve for *p*-nitrophenol was established: *p*-nitrophenol standards (0–120 μM final concentration) mixed with an equal volume of perchloric acid were injected onto the columnn (80 μL) without neutralization.

Enzyme kinetics

The formation of product was monitored over a range of substrate concentrations, after conditions of linearity for protein and time had been established at high and low substrate concentrations. Enzyme activities were expressed as nmol product formed/min/g tissue in order to relate directly the microsomal and cytosolic fractions. Median values for V_{max} and apparent K_m were calculated from substrate concentration and activity by the direct linear plot [9]. Results are expressed as means ± SEM. K_m values between tissues are compared by ANOVA with Tukey's HSD test.

RESULTS

Fluazifop-butyl esterase

Fluazifop-butyl was hydrolysed to fluazifop by microsomal and cytosolic fractions from rat liver, lung and skin as well as by plasma and red blood cells. Hydrolysis by all tissues followed Michaelis–Menten kinetics and V_{max} activities are shown in Table 1. Hydrolysis was greatest with liver microsomes and cytosol, about a tenth in lung and low in skin. Plasma esterase activity was half that in liver with little activity in the red blood cells.

Apparent K_m values for microsomal fluazifop-butyl esterase varied between 14 and 33 μM, with K_m values in the liver and the skin similar and significantly higher in the lung (Table 2). In the cytosol K_m values varied between 22 and 63 μM. The K_m for the lung was significantly higher than in the liver or skin.

Carbaryl esterase

Carbaryl was hydrolysed to 1-naphthol by

Table 1. The hydrolysis of a number of substrates by rat liver, lung and skin subcellular fractions, plasma and erythrocytes. Enzyme activity expressed as the V_{max}

		V_{max}			
		Fluazifop-butyl [μmol/min/g(mL)]	Carbaryl [nmol/min/g(mL)]	Paraoxon [nmol/min/g(mL)]	Phenylacetate [μmol/min/g(mL)]
Microsomes	Liver	6.29 ± 0.40	2.1 ± 0.25	333 ± 25	800 ± 75
	Lung	0.38 ± 0.10	1.6 ± 0.25	2.0 ± 0.25	4.86 ± 3.1
	Skin	0.02 ± 0.0015	0.2 ± 0.035	ND	1.13 ± 0.025
Cytosol	Liver	6.84 ± 0.85	6.7 ± 0.75	ND	30.7 ± 3.0
	Lung	1.50 ± 0.32	1.44 ± 0.36	ND	14.2 ± 5.5
	Skin	0.40 ± 0.06	0.5 ± 0.12	ND	3.44 ± 1.4
	Plasma	5.80 ± 0.48	3.00 ± 0.25	246 ± 12	290 ± 40
	Red blood cells	0.03 ± 0.015	ND	ND	ND

Values are means ± SEM (N = 6).
ND = not detectable.

Table 2. Apparent K_m values for the hydrolysis of a number of substrates by rat liver, lung, skin, plasma and erythrocytes

Substrate		K_m (μM) Fluazifop-butyl	Carbaryl	Paraoxon	Phenylacetate
Microsomes	Liver	14 ± 2.0	20 ± 3.5*	200 ± 8.3	1430 ± 140
	Lung	33 ± 5.5*	30 ± 3.5	380 ± 30*	1460 ± 580
	Skin	14 ± 2.8	32 ± 4.2	—	950 ± 100*
Cytosol	Liver	22 ± 4.5	53 ± 4.5*	—	900 ± 500
	Lung	63 ± 14*	26 ± 1.2	—	1330 ± 550
	Skin	42 ± 2.8	36 ± 3.8	—	340 ± 50*
	Plasma	18 ± 2.2	64 ± 4.0*	225 ± 15	2340 ± 400*
	Rbc's	33 ± 1.2	—	—	—

Values are means ± SEM (N = 6).
* $P < 0.05$ between tissues, within substrate, for microsomes or cytosol.

Table 3. Inhibition of fluazifop-butyl hydrolysis to fluazifop in rat liver, lung and skin subcellular fractions and plasma by 0.1 mM BNPP, physostigmine, $HgCl_2$ and paraoxon. Results are expressed as the % remaining activity of the control

		Substrate (μM)	Remaining activity (%) Control	BNPP	Physostigmine	Mercuric chloride	Paraoxon
Microsomes	Liver	300	100	33 ± 8	64 ± 5	60 ± 7.5	2 ± 0.25
	Lung	30	100	67 ± 6.5	100	—	10 ± 0.75
	Skin	20	100	40 ± 4.5	80 ± 2	—	17 ± 3
Cytosol	Liver	300	100	20 ± 2	69 ± 4.8	7 ± 1.2	2.1
	Lung	60	100	41 ± 2.5	95 ± 3.5	—	9 ± 6.5
	Skin	40	100	26 ± 2.5	86 ± 2.5	—	2 ± 2
	Plasma	200	100	63 ± 3	84 ± 2	100 ± 0	4 ± 0.85

Values are means ± SEM (N = 6).

microsomal and cytosolic esterase enzymes from the liver, lung, skin and plasma. The V_{max} for liver microsomal esterases was similar to the lung and about 10 times that of the skin (Table 1). Cytosolic carbaryl esterase activity was greater than microsomal in the liver and skin and similar in the lung.

The apparent K_m values for microsomal carbaryl esterase varied between 20 and 32 μM, with K_m values in the liver significantly lower than in the lung and skin. The apparent K_m for cytosolic carbaryl esterase varied between 26 and 53 μM, with K_m values in the liver significantly higher than in lung and skin, but similar to that of the plasma (Table 2). Microsomal esterases in general had higher affinity for carbaryl than the cytosolic esterases.

Paraoxonase

Paraoxonase activity was detected only in the microsomes and the plasma. There was 160 times greater activity in the liver microsomes than in the lung, whereas in the skin microsomes and the red blood cells no activity was detected [limit of detection > 0.3 nmol/min/g(mL)]. Values for V_{max} can be seen in Table 1.

The apparent K_m values for paraoxonase varied between 200 and 380 μM, with the liver microsomes similar to plasma but significantly lower than the lung (Table 2).

Phenylacetate esterase

Phenylacetate was hydrolysed by both microsomes and cytosol from liver, lung, skin and plasma (V_{max} values are shown in Table 1). Hydrolysis by liver microsomes was twice plasma, 160 times the lung and 700 times the skin. Cytosolic activity was generally lower.

The apparent K_m for phenylacetate esterase varied between 0.95 and 1.46 mM (microsomal) and between 0.34 and 1.33 mM (cytosolic). The K_m value for the skin was significantly lower than that of the liver and lung. The apparent K_m value for plasma was found to be significantly higher than liver or lung (Table 2).

Inhibition of hydrolysis of fluazifop-butyl, with microsomal and cytosolic fractions from all tissues was greater than 80% with paraoxon. Inhibition by BNPP was more variable, activity remaining ranged from 20 to 67%. Physostigmine inhibition was less (0–36%). Mercuric chloride did not inhibit plasma hydrolysis but strongly inhibited hydrolysis by liver cytosol (Table 3).

Similarly, inhibition of hydrolysis of carbaryl by BNPP varied between 76 and 20% and by physostigmine varied between 3.5 and 12% depending on tissue and fraction (Table 4).

Inhibition of hydrolysis of phenylacetate by paraoxon was less than for fluazifop-butyl varying between 27 and 67% depending on tissue. BNPP inhibition varied from 0 to 35% whereas inhibition due to physostigmine varied from 11 to 40%, with the exception of the lung cytosol which showed an inhibition of 96%. There was a wide range in

Table 4. Inhibition of carbaryl hydrolysis to 1-naphthol in rat liver, lung and skin subcellular fractions and plasma by 0.1 mM BNPP and physostigmine. Results are expressed as the % remaining activity of the control

		Substrate (μM)	Remaining activity (%)		
			Control	+BNPP	+Physostigmine
Microsomes	Liver	25	100	72 ± 3	96 ± 2
	Lung	25	100	59 ± 4	74 ± 5
	Skin	25	100	24 ± 7.5	89 ± 5
Cytosol	Liver	25	100	80 ± 4.2	97 ± 1.8
	Lung	25	100	70 ± 6.2	97 ± 1.5
	Skin	25	100	64 ± 8.8	88 ± 7
	Plasma	25	100	56 ± 3.5	93 ± 2.5

Values are means ± SEM (N = 4).

Table 5. Inhibition of phenylacetate hydrolysis by rat liver, lung and skin subcellular fractions and plasma by 0.1 mM BNPP, mercuric chloride, physostigmine and paraoxon. Results are expressed as the % remaining activity of the control

		Substrate (mM)	Control	Remaining activity (%)			
				BNPP	Mercuric chloride	Physostigmine	Paraoxon
Microsomes	Liver	2	100	84 ± 3.5	60 ± 16	87 ± 1	50 ± 3.8
	Lung	2	100	94 ± 5	85 ± 12	81 ± 5	37 ± 8.5
	Skin	2	100	100	73 ± 14	60 ± 10	38 ± 8.6
Cytosol	Liver	2	100	75 ± 12	93 ± 1.2	57 ± 14	33 ± 7.5
	Lung	2	100	100	62 ± 17	4 ± 2.5	48 ± 5
	Skin	2	100	65 ± 5	30 ± 10	78 ± 1.8	35 ± 5
	Plasma	2	100	81 ± 4	59 ± 2.5	89 ± 4	83 ± 5

Values are means ± SEM (N = 6).

inhibition by mercuric chloride from 7% with skin microsomes to 70% with skin cytosol (Table 5).

DISCUSSION

The inner-tissue distribution of carbaryl and fluazifop-butyl esterase activity in the rat was similar. Most activity was found in the liver and plasma, however there was a significant amount of activity in the skin and lung. In the liver, most hydrolysis of carbaryl took place in the cytosol, whereas fluazifop-butyl hydrolysis was equally distributed between the microsomal and cytosolic fractions. In the endoplasmic reticulum, carbaryl can be metabolized both by hydrolysis to naphthol and by microsomal oxidation to 4 and 5 hydroxycarbaryl [5] whereas in the cytosol, carbaryl metabolism is entirely due to hydrolysis. In contrast, fluazifop-butyl is only metabolized by hydrolysis. The apparent K_m values for hydrolysis of fluazifop-butyl and carbaryl in the different tissues studied were similar, suggesting the involvement of similar esterases.

To characterize the enzymes involved, inhibitory studies were carried out using differential inhibitors. paraoxon completely inhibited cholinesterase and carboxylesterase (B esterases), BNPP had been shown to be a differential inhibitor of carboxylesterase isoenzymes in the liver [10] and lung [11] and physostigmine, an anticholinesterase, inhibited cholinesterase and to some extent carboxylesterase. Mercuric chloride inhibited enzymes which contain an −SH group (cysteine) at the active centre such as A esterases and arylesterases.

Paraoxon caused almost complete inhibition of fluazifop-butyl and carbaryl hydrolysis by both microsomal and cytosolic fractions and plasma indicating involvement of B esterases. Significant inhibition by BNPP but less effect by physostigmine suggested the involvement of carboxylesterases in rat liver, lung, skin microsomes and cytosol. Plasma esterase activity was inhibited by BNPP but not significantly by physostigmine, consistent with involvement of carboxylesterases present in rat plasma. In the plasma, no inhibition of fluazifop-butyl hydrolysis by mercuric chloride was seen, indicating that arylesterases were not involved; however, the high inhibition with liver cytosol did not fit with the other inhibition results.

There is very little information on the classification of cytosolic esterases, most studies have concerned microsomal carboxylesterase [12]. Results in this study indicate that xenobiotic metabolizing carboxylesterase activity is also present in the cytosol. Differential inhibition studies with BNPP and specific substrates have indicated at least four carboxylesterase isoenzymes in liver [10] and two isoenzymes in lung [11]. Gaustad *et al.* [11] found a carboxylesterase isoenzyme (pI < 4.2) in guinea pig lung which was resistant to BNPP inhibition. The carboxylesterase isoenzymes involved in fluazifop-butyl and carbaryl hydrolysis have not been identified yet, these may be tissue specific thus producing inter-tissue differences in inhibition profiles.

The distribution of paraoxon hydrolysing enzymes in the rat was different from the distribution of carbaryl and fluazifop-butyl esterase. Most

paraoxonase activity was found in the liver microsomes and the plasma. A very small amount of paraoxonase activity was detected in the lung microsomes and none in the skin. No activity was found in the cytosolic fraction of the tissues studied. Paraoxon has previously been shown to be hydrolysed by an 'A' esterase in liver and plasma.

Paraoxonase and arylesterase have been classified separately [2], however, there has been controversy as to whether phenylacetate and paraxon are hydrolysed by the same enzyme in human plasma [13]. Most phenylacetate esterase activity was detected in the liver microsomes and the plasma of the rat with a similar distribution to paraoxonase, with a smaller amount in the lung and skin. There was, however, some activity in the cytosolic fractions although no paraoxonase was detected. The lack of paraoxon hydrolysis in the cytosol, where carboxylesterase is present, suggests that binding of paraoxon to carboxylesterase is not responsible for the release of the leaving group *p*-nitrophenol. Phenylacetate has been regarded as a marker for arylesterase activity, however, the inhibition profile in the cytosol suggested involvement of carboxylesterases rather than arylesterases.

Extrahepatic hydrolysis may have different degrees of importance *in vivo* depending on the route of absorption. Following dermal exposure *in vivo* in the rat hydrolysis could occur locally in the skin and also in the blood prior to hepatic hydrolysis. However, Clark *et al.* [14] have shown that during percutaneous absorption of fluazifop-butyl in an *in vitro* absorption system with viable rat skin, hydrolysis was less than predicted from incubation studies. In humans, occupational exposure to fluazifop-butyl is principally by absorption through the skin. Humans have little plasma carboxylesterase, and fluazifop-butyl is a poor substrate for human plasma cholinesterase, so the liver appears to be the major site of metabolism [15].

Similarly, following dermal absorption of carbaryl the skin may be important in carbaryl metabolism. The capacity of skin homogenates to metabolize carbaryl may also not be fully utilized during absorption, at least in *in vitro* models of absorption [6].

Rat skin and lung tissue had little capacity to hydrolyse paraoxon. Plasma and liver paraoxonase are the major sites for hydrolysis *in vivo* and play the most important role in the detoxification of paraoxon.

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