Toxicology, 65 (1990) 61—74
Elsevier Scientific Publishers Ireland Ltd.

Absorption, distribution, excretion and metabolism of a single oral dose of [^{14}C]tri-*o*-cresyl phosphate (TOCP) in the male rat

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(Received March 12th, 1990; accepted August 29th, 1990)

Summary

A single oral dose of 50 mg/kg of [^{14}C]TOCP was administered in corn oil to male rats. Three animals were sacrificed at each of 2, 6 and 12 h and 1, 2 and 5 days following dosing, and tissues and excreta were analyzed for ^{14}C. Within 5 days, 63 and 36% of the dose were recovered in the urine and feces, respectively. Initially, the highest concentrations of radioactivity were observed in the gastrointestinal tract, its contents, the urinary bladder, liver and kidneys. Appreciable concentrations of ^{14}C were detected in plasma, red blood cells, lungs and adipose tissues, while neural tissues, muscle, spleen and testes contained lower concentations of radioactivity. Among neural tissues, the sciatic nerve contained the highest concentrations of ^{14}C at all time points studied. The concentration of TOCP in plasma was at maximum by 6 h then declined biexponentially with terminal half-life of 46 h. The predominant metabolites in plasma were *o*-cresyl dihydrogen phosphate, di-ocresyl hydrogen phosphate and *o*-hydroxybenzoic acid (salicylic acid). Small concentrations of the neurotoxic metabolite of saligenin cyclic-*o*-tolyl phosphate, were detected in plasma at all but the last time point analyzed. Most of the radioactivity extracted from the livers of rats sacrificed at 2 and 4 h were metabolites. No TOCP was detected in the urine or feces collected within 3 days after dosing. The major metabolite in the urine and feces was *o*-cresyl dihydrogen phosphate followed by di-*o*-cresyl hydrogen phosphate, salicylic acid, *o*-hydroxybenzyl alcohol and *o*-cresol. This study supports the hypothesis that the insensitivity of the rat to TOCP-induced delayed neurotoxicity may be attributed, in part, to the disposition and metabolism of this chemical.

Key words: Tri-*o*-cresyl phosphate; Organophosphorus compound-induced delayed neurotoxicity; Metabolism; Rat; Delayed neurotoxicity

Introduction

Tri-*o*-cresyl phosphate (TOCP) is an industrial chemical used as a plasticizer in lacquers and varnishes [1]. TOCP is among the group of organophosphorus

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0300-483X/90/$03.50

compounds known to induce the toxic condition described as organophosphorus compound-induced delayed neurotoxicity (OPIDN) [2—4]. Humans and some animal species (chickens, cats, dogs and cows) are sensitive to this toxic effect, while rodents (rats and mice) are less sensitive and some non-human primates are not sensitive [3—4]. Since 1920, approximately 40 000 human cases of OPIDN have been attributed to TOCP [3,4]. Clinical signs appear after 6—14 days as ataxia which may progress to paralysis; this is joined by a Wallerian-type degeneration of axons and myelin of both central and peripheral nervous systems [3,5]. Sensitivity to OPIDN is related to age since chicks and kittens are more resistant than their adult counterparts [6,7].

TOCP is absorbed from the alimentary canal of cats and dogs [8], human palm skin, the abdominal skin of the dog [9] and the skin of the cat [10—12]. Smith et al. [8] identified *o*-cresol in the urine of cats and dogs treated with TOCP. Further studies showed that TOCP is metabolized to a more potent esterase inhibitor identified as saligenin cyclic-*o*-tolyl phosphate [2-(*o*-cresyl)-4*H*-1,3,2-benzodioxaphosphorane-2-one] [10—15]. Saligenin cyclic-*o*-tolyl phosphate is believed to be the neurotoxic metabolite of TOCP since it is five times as neurotoxic as TOCP to the hen [16].

Perry et al. [17] studied the pharmacokinetic parameters of TOCP in male, Fischer 344 rat following oral administration of 2, 20, or 200 mg/kg. Rats receiving TOCP at 2 or 20 mg/kg eliminated 90% of the dose (60—70% urine; 20—25% feces), while at 200 mg/kg only 60% of the dose was eliminated (45% urine, 16% feces) by 3 days. Nomeir and Abou-Donia [10,11] reported on the metabolism and disposition of [^{14}C]TOCP in the male cat following dermal application of 50 mg/kg. The major metabolites in the urine and feces were *o*-cresol, di-*o*-cresyl hydrogen phosphate and *o*-cresyl dihydrogen phosphate. In plasma, di-*o*-cresyl hydrogen phosphate and *o*-cresyl dihydrogen phosphate were the major metabolites, while saligenin cyclic-*o*-tolyl phosphate was present in smaller amounts. TOCP was the predominant compound in the brain, spinal cord and sciatic nerve, while the majority of the ^{14}C found in the liver, kidney, and lung was in the form of metabolites [10]. The major metabolite identified in the liver, kidney and lung was salicylic acid followed by di-*o*-cresyl hydrogen phosphate, whereas di-*o*-cresyl hydrogen phosphate and *o*-cresyl hydrogen phosphate were the predominant metabolites in the brain, spinal cord and sciatic nerve. A similar study on the metabolism and pharmacokinetics of a single oral 50 mg/kg [^{14}C]TOCP was carried out in hens [18,19]. TOCP was slowly eliminated in the fecal-urinary excreta [18]. TOCP disappearance from the plasma followed monoexponential kinetics with a half-life of 2.2 days. Significant concentrations of the active metabolite saligenin cyclic-*o*-tolyl phosphate was detected in the plasma and other tissues [19].

The present report describes the absorption, distribution, excretion and metabolism of TOCP in the male rat following the oral administration of 50 mg of the ^{14}C-labeled compound/kg of body weight. This dose was selected so that it does not affect metabolic processes since it neither produces acute cholinergic nor delayed neurotoxic effects in the rat [20].

Materials and methods

Chemicals

TOCP (Phenyl U-^{14}C), specific activity of 4.83 μCi/μmol and radiochemical purity of ≥98% was obtained from Midwest Research Institute, Kansas City, MO. Unlabeled TOCP was obtained from Eastman Kodak Co., Rochester, NY. Amberlite XAD-4 was obtained from Supelco, Inc., Bellefonte, PA. The sources of TOCP metabolites and other chemicals used in this study were described elsewhere [10—12]. All solvents used were HPLC grade and were purchased from Fisher Scientific (Raleigh, NC). Other chemicals were obtained in the highest purity available.

Animals

Male Sprague—Dawley rats, with an average body weight of 275 g, (6 month old) were obtained from Charles River. The animals were housed in a temperature-controlled (21—23 °C) room with a 12-h light/dark cycle before and during the study. They were allowed to adapt to the environment for 1 week prior to dosing. The animals were supplied with feed (Rodent Laboratory Chow, Ralston, Purina Co., St. Louis, MO) and water ad libitum.

Treatment of animals

Each rat received a single p.o. dose of 50 mg (6.68 μCi)/kg of body weight in corn oil (0.75 ml/kg). Treated rats were held in individual glass metabolism cages that allowed separate collection of urine and feces. Expired air was passed through two consecutive traps, the first contained 50 g of Amberlite XAD-4 to collect volatile chemicals and the second contained 250 ml of a 1:2 mixture of ethanolamine/ethylene glycol monomethyl ether to collect CO_2. Three treated rats were sacrificed at each of 2, 6 and 12 h and 1, 2 and 5 days. The animals were anesthetized with ether and killed by exsanguination. Blood-samples were centrifuged at 2000 *g* for 15 min to separate the plasma from the red blood cells. Individual tissues were excised, weighed and samples were taken for total radioactivity analysis. Muscle and adipose tissues were sampled from various locations. The tissues were stored at −25 °C until extraction and analysis. Estimates of tissue weights as percentages of total body weight for an adult rat obtained from the literature were as follows: muscle 30%, plasma 4%, red blood cells 6% and adipose 7% [21,22].

Analysis of radioactivity

Radioactivity in tissue, body fluids, excreta and traps were analyzed as described earlier [10,11] using tissue oxidizer in combination with liquid scintillation counting.

Analysis of TOCP and its metabolites

High performance liquid chromatography (HPLC) was utilized to separate TOCP and its metabolites from various biological samples [23]. Briefly, TOCP and its metabolites were separated using a C_{18} cartridge fitted into an RCM-100

radial compression separation system. The mobile phase was a linear gradient of 25— 80% acetonitrile in 2% aqueous acetic acid in 22 min at a solvent flow rate of 1.3 ml/min. The compounds were detected by monitoring the ultraviolet absorbance of the column eluates at 254 nm. A mixture of authentic standards of TOCP and nine of its metabolites were injected in the HPLC along with samples to be analyzed. The radioactivity eluted from the HPLC was collected in scintillation vials and analyzed by liquid scintillation counting. Analysis of each sample was carried out in triplicate unless otherwise mentioned. Recovery of the radioactivity from the HPLC was almost complete (>98%) in all samples analyzed. Other HPLC solvent systems were used to confirm the identity of TOCP and its metabolites [23]. All samples were filtered through a 0.45-μm filter prior to HPLC analysis. TOCP and its various metabolites were found to be stable under the conditions of extraction and analysis. This was confirmed by spiking tissues of untreated rats with TOCP and its metabolites which were then similarly extracted and analyzed.

Plasma

TOCP and its metabolites present in plasma of treated rats were extracted with ether as previously described [23].

Urine

Urine collected in days 1, 2 and 3 after dosing (95% of the urinary radioactivity) was individually analyzed without prior extraction. Samples of urine excreted within 24 h after dosing were also incubated in 0.1 M acetate buffer (pH 5) for 24 h at 37°C with or without sulfatase or β-glucuronidase (approx. 300 units of β-glucuronidase or 40 units of sulfatase/ml urine). Following incubation, an equal volume of methanol was added, centrifuged and analyzed by high performance liquid chromatography and liquid scintillation counting (HPLC-LC).

Feces

Daily feces excreted from two 5-day rats were pooled for all days and extracted with acetonitrile 4 × 200 ml. The extract was dried with anhydrous $MgSO_4$ and concentrated under vacuum prior to HPLC-LC analysis.

Liver

Livers from rats sacrificed at 2 and 6 h after dosing were extracted with acetonitrile as described earlier [10,11]. Extracts were analyzed by HPLC-LC.

Kinetic analysis

TOCP concentration versus time was plotted on semilogarithmic papers. The β value was obtained by linear regression of the terminal exponential decline in TOCP concentration, using the expression $t_{1/2} = 0.693/\beta$.

TABLE I

CONCENTRATION OF RADIOACTIVITY (μg TOCP EQUIVALENT/g TISSUE OR ml PLASMA) IN VARIOUS TISSUES OF MALE RATS FOLLOWING A SINGLE ORAL ADMINISTRATION OF 50 mg OF [^{14}C]TOCP/kg OF BODY WEIGHT

Tissue	Time after dosing[a]					
	2 h	6 h	12 h	1 day	2 days	5 days
Brain	0.70 ± 0.21	1.12 ± 0.16	1.12 ± 0.78	0.19 ± 0.08	0.07 ± 0.04	0.05 ± 0.03
Spinal cord	1.05 ± 0.29	0.55 ± 0.07	0.48 ± 0.27	0.66 ± 0.47	0.05 ± 0.03	0.03 ± 0.02
Sciatic nerve	3.39 ± 0.51	2.25 ± 0.25	2.45 ± 0.52	2.00 ± 0.32	0.98 ± 0.22	0.10 ± 0.08
Plasma	6.03 ± 0.43	8.24 ± 0.42	5.54 ± 0.62	5.36 ± 0.78	3.19 ± 0.49	1.51 ± 0.35
Red blood cells	2.14 ± 0.23	4.23 ± 0.99	4.38 ± 1.50	4.31 ± 0.19	5.23 ± 1.16	3.13 ± 0.72
Liver	15.00 ± 4.70	16.10 ± 1.88	13.60 ± 3.70	9.97 ± 0.41	7.59 ± 1.16	4.73 ± 0.19
Kidney	8.02 ± 1.04	12.40 ± 1.61	9.35 ± 3.10	5.38 ± 0.50	3.89 ± 0.51	1.66 ± 0.05
Lungs	4.14 ± 0.49	6.24 ± 1.52	4.61 ± 1.16	3.14 ± 0.23	1.83 ± 0.60	1.34 ± 0.37
Heart	2.35 ± 0.26	2.66 ± 0.44	2.14 ± 0.70	1.49 ± 0.10	1.19 ± 0.28	0.47 ± 0.26
Spleen	1.83 ± 0.23	2.64 ± 0.36	1.79 ± 0.42	1.27 ± 0.20	1.12 ± 0.27	0.20 ± 0.16
Muscle	0.85 ± 0.21	0.87 ± 0.09	0.60 ± 0.43	0.83 ± 0.13	0.45 ± 0.21	0.30 ± 0.18
Adipose tissue	2.16 ± 0.42	7.50 ± 3.01	4.61 ± 0.73	4.32 ± 1.40	2.85 ± 1.27	0.97 ± 0.31
Skin	5.40 ± 1.22	7.60 ± 2.06	22.00 ± 9.61	53.10 ± 20.00	4.43 ± 1.91	1.91 ± 1.14
Testes	1.01 ± 0.05	1.91 ± 0.33	1.17 ± 0.37	0.96 ± 0.13	0.70 ± 0.13	0.48 ± 0.23
Urinary bladder	23.90 ± 10.60	155.00 ± 68.90	29.50 ± 15.40	20.50 ± 12.40	4.85 ± 1.30	1.12 ± 0.42
Stomach	498.00 ± 109.00	40.20 ± 23.00	13.20 ± 1.22	4.18 ± 0.39	2.06 ± 0.59	0.80 ± 0.07
Small intestine	64.50 ± 7.38	62.80 ± 14.40	89.80 ± 50.60	10.50 ± 0.47	4.20 ± 0.47	0.72 ± 0.23
Large intestine	2.78 ± 0.40	10.70 ± 3.14	48.90 ± 25.70	17.30 ± 6.79	5.81 ± 2.39	0.46 ± 0.07

[a]Results are the average of 6 determinations from three different rats ± standard deviation (n = 3).

Results

Disposition of radioactivity

TOCP was readily absorbed from the gastrointestinal (GI) tract of the rat and subsequently distributed throughout the body. Radioactivity was detected in all tissues analyzed by 2 h after dosing (Table I).

Concentration of ^{14}C. At 2 h, the highest concentrations of ^{14}C were observed in the stomach, small intestine, urinary bladder, liver, kidney, and plasma, while the lowest concentrations were detected in the brain, spinal cord, muscles and testes. The radioactivity concentration in most tissues plateaued from 6 to 12 h while it sharply increased in the urinary bladder (> 6-fold) and to a smaller extent in the plasma, red blood cells, lungs, kidneys, testes, adipose tissue and the lower parts of the GI tract. The concentration of ^{14}C in the stomach decreased sharply (> 12-fold) by 6 h following administration. By 12 h the concentration of radioactivity decreased in the urinary bladder (> 5-fold) and increased in the lower parts of the GI tract, while smaller changes were observed in the rest of the tissues. By 24 h, there was a general slight decrease in radioactivity concentration in some tissues while a greater decline was observed in various parts of the GI tract. The concentration of ^{14}C remained almost unchanged in plasma, red blood cells and adipose tissue. By 48 h the ^{14}C concentration in most tissues marginally decreased, while the drop was relatively low in plasma, red blood cells, liver, spleen, kidney and testes. By 5 days, the ^{14}C concentration dropped significantly in all tissues analyzed. By this time point, the highest concentrations of radioactivity were observed in the liver, red blood cells, skin, kidney, and lung. Among neural tissues, the sciatic nerve contained the highest concentration of ^{14}C (Table I).

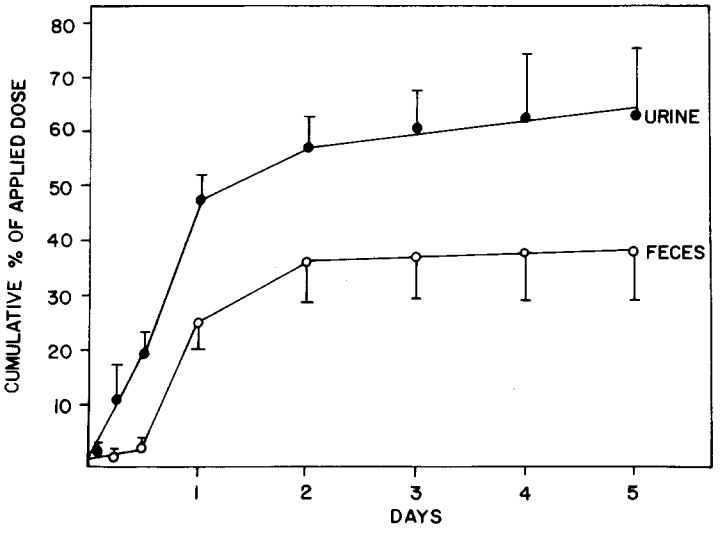

Fig. 1. Cumulative excretion of radioactivity in the urine and feces of male rats treated orally with 50 mg of [^{14}C]TOCP/kg of body weight.

Total ^{14}C. To study the fate of TOCP in the rat, the ^{14}C in each tissue was determined. In the first 12 h the majority of radioactivity was located in the GI tract and its contents. Tissues with larger masses such as muscle, skin and liver contained higher contents of ^{14}C. The total ^{14}C In the GI tract remained relatively high in the first 12 h then decreased by 24 h and continued to decline to almost negligible levels by 5 days. By 2 h the total of ^{14}C in tissues was approximately 13% of the dose, which remained almost unchanged until 12 h and then dropped to approximately 7% by 24 h. The total of ^{14}C in tissues dropped to approximately 3.2 and 1.5% of the dose by 2 and 5 days, respectively (data not shown).

Excreted ^{14}C. TOCP-derived radioactivity was excreted in the urine and feces. By 5 days, 63% and 36% of the dose were excreted in the urine and feces, respectively (Fig. 1). Most of the radioactivity was excreted between 6 and 24 h with the maximum rate of excretion occurring between 12 and 24 h. Very little, if any, radioactivity was observed in the CO_2 or the Amberlite XAD-4 traps.

TOCP and metabolites

Plasma. Plasma was extracted and analyzed by HPLC-LS. Figure 2 illustrates the concentration of TOCP in plasma as a function of time. At 6 h TOCP concentration was approximately twice of that at 2 h (Fig. 2). By 5 days TOCP

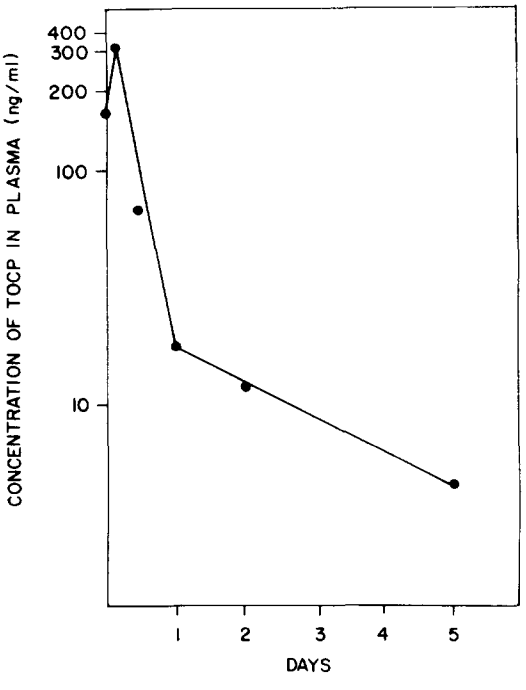

Fig. 2. Concentration of TOCP in the plasma of male rats following the oral administration of 50 mg of [^{14}C]TOCP/kg of body weight.

TABLE II

CONCENTRATIONS (ng/ml) OF TOCP METABOLITES IN THE PLASMA OF MALE RATS FOLLOWING ORAL ADMINISTRATION OF 50 mg OF [^{14}C]TOCP/kg OF BODY WEIGHT[a]

Compound	2 h	6 h	12 h	1 day	2 days	5 days
o-Cresyl dihydrogen phosphate	276.00	56.00	170.00	61.00	0	0
Di-*o*-cresyl hydrogen phosphate	181.00	122.00	94.00	82.00	0	8.2
o-Cresol	2.90	46.00	29.00	40.00	1.10	1.30
o-Hydroxybenzyl alcohol	26.00	28.00	39.00	20.00	5.00	3.30
Salicylaldehyde	10.00	21.50	16.90	4.60	1.90	0.00
Salicylic acid	133.00	109.00	124.00	43.00	10.40	3.30
Saligenin cyclic-*o*-tolylphosphate	11.20	14.00	12.50	4.60	4.20	0
Hydroxymethyl TOCP	4.90	12.40	12.00	9.50	0.50	0
Unknowns[b]	115.00	100.00	147.00	51.00	25.00	14.60

[a] Each time point represents the average of 2 replicates from plasma pooled from three rats.

[b] Unknowns represent radioactivity which did not cochromatograph with any of the authentic standards.

level in plasma dropped to approximately 1% of that observed at 6 h. The decline of TOCP in plasma followed a biexponential kinetics with apparent terminal half-life of 46 h.

Table II presents the concentrations levels of TOCP metabolites in plasma at various time points. *o*-Cresyl dihydrogen phosphate, di-*o*-cresyl hydrogen phosphate and salicylic acid were the predominant metabolites followed by *o*-cresol and *o*-hydroxybenzyl alcohol while saligenin cyclic *o*-tolyl phosphate, salicylaldehyde and hydroxymethyl TOCP were present in relatively smaller amounts.

Tissues. Radioactivity present in the liver of rats sacrificed at 2 and 6 h were extracted and analyzed. Approximately 30% of the radioactivity present in the liver was extractable. The concentration of TOCP in the liver increased between 2 and 6 h (Table III). All metabolites identified in the plasma except hydroxymethyl TOCP were also identified in the liver of treated rats (Table III). Radioactivity extracted from other tissues was too low to be analyzed. Non-extractable radioactivity might have been bound TOCP metabolites to the liver macromolecules.

Urine. Table IV presents various metabolites present in the urine excreted within the first 3 days following administration. Urine from each day was analyzed separately. There was no apparent difference in the metabolic profile of TOCP in the urine as a function of time. *o*-Cresyl dihydrogen phosphate accounted for the majority of radioactivity present in the urine. Di-*o*-cresyl hydrogen phosphate, *o*-hydroxybenzyl alcohol and salicylic acid were present in almost equal amounts while the levels of *o*-cresol and salicylaldehyde were relatively low. TOCP, saligenin cyclic-*o*-tolyl phosphate and hydroxymethyl TOCP were not detected in the urine.

TABLE III

CONCENTRATIONS OF (ng/g fresh liver)[a] TOCP AND METABOLITES IN LIVER EXTRACTS[b] OF RATS TREATED WITH A SINGLE ORAL DOSE OF 50 mg OF [^{14}C]TOCP/kg OF BODY WEIGHT[a]

Compound identified	2 h	6 h
o-Cresyl dihydrogen phosphate	898	957
Di-*o*-Cresyl hydrogen phosphate	1479	979
o-Hydroxybenzyl alcohol	500	727
Salicylic acid	354	270
o-Cresol	198	422
Saligenin cyclic-*o*-tolyl phosphate	203	54
TOCP	135	480
Unknowns[c]	1260	1344

[a] Each value is the average of 2 replicates from combined livers of 3 rats.

[b] Radioactivity extracted from liver is 30% of the total ^{14}C present.

[c] Unknowns correspond to radioactivity which did not cochromatograph with any of the standards used, concentrations are expressed as TOCP equivalent.

TABLE IV

URINARY METABOLITES OF TOCP FROM RATS TREATED WITH A SINGLE ORAL DOSE OF 50 mg OF [^{14}C]TOCP/kg OF BODY WEIGHT[a]

Compound identified	% of radioactivity excreted[b]		
	Day 1	Day 2	Day 3
o-Cresyl dihydrogen phosphate	78.6 ± 3.9	77.8 ± 2.8	76.1 ± 2.8
Di-*o*-cresyl hydrogen phosphate	3.2 ± 0.4	3.9 ± 2.5	3.6 ± 2.2
o-Hydroxybenzyl alcohol	3.4 ± 0.3	2.7 ± 1.4	1.5 ± 1.3
Salicylic acid	2.0 ± 0.8	1.1 ± 0.9	1.7 ± 1.5
o-Cresol	2.2 ± 1.3	1.1 ± 0.8	1.7 ± 0.7
Salicylaldehyde	0.4 ± 0.1	0.4 ± 0.2	0.7 ± 0.2
Unknowns[c]	9.6 ± 2.1	11.8 ± 1.5	15.0 ± 4.1

[a] Results are the average of 6 replicates from 3 different (n = 3) rats ± S.D.
[b] Radioactivity excreted in days 1—3 is 96% of the total urinary radioactivity.
[c] Unknowns are described in the footnotes of previous Tables.

Urine collected within 24 h after dosing was incubated with β-glucuronidase or sulfatase then analyzed by HPLC-LS. Very little, if any, increase in the relative percentages of *o*-hydroxybenzyl alcohol and *o*-cresol were observed while no changes were observed for the rest of the urinary metabolites. These results suggest that very little, if any, sulfates and glucuronides are present in the urine.

Feces. Feces of treated rats excreted within 5 days were combined, extracted and analyzed by HPLC-LS. Approximately 38% of the radioactivity excreted in the feces was extracted with acetonitrile. Table V shows that *o*-cresyl dihydrogen phosphate and di-*o*-cresyl hydrogen phosphate were the predominant metabolites identified in the feces followed by *o*-hydroxybenzyl alcohol, salicylic acid and *o*-cresol.

TABLE V

FECAL METABOLITES OF TOCP FROM RATS TREATED WITH A SINGLE ORAL DOSE OF 50 mg OF [^{14}C]TOCP/kg OF BODY WEIGHT[a]

Compound	% of radioactivity extracted from the feces[a]
o-Cresyl dihydrogen phosphate	36 ± 11
Di-*o*-Cresyl hydrogen phosphate	33 ± 4
o-Hydroxybenzyl alcohol	11 ± 4
Salicylic acid	6 ± 2
o-Cresol	2 ± 0.2
Unknowns	11 ± 3.0

[a] Results are the average of 4 replicates from 2 different rats.
[b] Approximately 40% of the fecal radioactivity was extractable.

Discussion

TOCP was readily absorbed from the alimentary canal of the male rat and subsequently distributed throughout the body. Initially the highest concentrations of ^{14}C were associated with the routes of entry and excretion, while the lowest were detected in the brain, spinal cord, muscle and testes. The levels of ^{14}C in most tissues remained relatively unchanged by 12 h, while by 24 h, ^{14}C levels decreased in most tissues, as a result of metabolism and excretions. By 2 and 5 days, the concentration of ^{14}C dropped in all tissues. The approximate half-life of radioactivity in tissues was 1 day.

TOCP-derived radioactivity was excreted mainly in the urine and to a lesser extent in the feces. By 5 days following dosing virtually all of the administered radioactivity was recovered in the urine (63% of the dose) and feces (36%). Most of the radioactivity was voided in the first day (72% of the dose), and by 2 days 94% was recovered. These results are in agreement with those of Perry et al.[17] who indicated that 90% of the 2 or 20 mg/kg dose of TOCP was excreted in the urine (60—70% of the dose) and feces (20—25%) of Fischer 344 rats within 3 days after dosing. The low urinary excretion of tri-*o*-cresyl hydrogen phosphate, which exhibited a relatively high plasma concentration, suggests that it was hydrolyzed prior to its excretions as indicated by the presence of high concentration of *o*-cresyl dihydrogen phosphate, its hydrolytic product, in the urine.

Upon comparing the results of tissue distribution with another study in hens treated with an equal oral dose [18,19], some worthwhile observations are noted. In both species the peak level of ^{14}C was reached in most tissues 12 h after dosing. At this time, most of the rat tissues contained more radioactivity than those of the hen. Among nervous tissues, the brain and sciatic nerves in the rat had higher levels of radioactivity (3-, and 2-fold respectively) while the spinal cord contained less activity (0.65-fold) in the rat than the chicken.

However, in the hen the rate of disappearance of radioactivity from most tissues was slower relative to that of the rat. By 5 days following administration, hen nervous tissue contained higher levels of ^{14}C in the brain (3-fold), spinal cord (10-fold) and sciatic nerve (5-fold) and to a lesser degree for the rest of the tissues. These differences in the rate of disappearance of TOCP-derived radioactivity between the rat and the chicken may be an important contributing factor to the difference in sensitivity to OPIDN observed between the two species. The rat (less sensitive) appears to be more capable of clearing TOCP from nervous tissues at faster rates compared to the chicken (sensitive species). Other factors contributing to the low sensitivity of the rat to OPIDN may be differences in the delayed neurotoxicity target proteins and/or the rapid repair of the nervous tissues in the rat compared to the chicken.

TOCP was detected in the plasma of treated rats at all time points analyzed (Fig. 2). It is noteworthy, that TOCP plasma concentration in rats was far lower (approx. 6—13-fold lower) at 24 h and all time points thereafter than that of the chicken [18]. Also, the $t_{1/2}$ of TOCP was 53 h in chicken plasma [19] compared to 46 h in the rat. More importantly, although saligenin cyclic-*o*-tolyl phosphate,

the neurotoxic metabolite of TOCP, was detected in rat plasma at points 2 h to 2 days, its concentrations in rats were far less than these observed in plasma of TOCP-treated hens (for example, 13-, 46- and 65-fold difference at 12, 24 and 48 h, respectively and undetected vs. 56 ng/ml at 5 days) in rats and hens, respectively [19]. Also, the plasma half-lives of this metabolite were 18 h and 49 h in the rat and hen, respectively.

Kurebayashi et al., [24] studied the metabolism and disposition of tri-*p*-cresyl phosphate (TPCP), a non-neurotoxic isomer of TOCP, in male Wistar rats. Their study showed that in the rat TPCP was subjected to oxidation and dearylation reactions similar to what was observed in this and other studies conducted with TOCP in our laboratory. TPCP is not neurotoxic, however, probably because it can not cyclize to form the neurotoxic metabolite [24].

Previous studies with other neurotoxic chemicals have shown that pharmacokinetics and metabolism may play a prominent role in species sensitivity to OPIDN [For reviews see 25 and 26]. Studies with EPN (*O*-ethyl *O*-*p*-nitrophenyl phenylphosphonothioate) and its oxon, EPNO (*O*-ethyl *O*-*p*-nitrophenyl phenylphosphonate) showed that, in vitro, rat liver microsomes had a greater capacity to metabolize these neurotoxic chemicals to non-neurotoxic products than chicken liver microsomes [27]. In vivo, a series of studies on EPN metabolism in the rat, cat, and hen indicated that EPN was readily metabolized and excreted from the rat, while it was more persistent in the sensitive species chicken and cat [28—32]. Also comparing the levels of EPN-derived radioactivity in tissues of chicken given subneurotoxic dose (4 mg/kg) to those of birds given neurotoxic dose (50 mg/kg) showed that disproportionally higher levels of residues were found in tissues of hens given the toxic dose (dose ratio is 12.5-fold). Fat showed the highest increase (1500-fold) followed by lungs (340-fold), while most other tissues had between 28—123-fold increase in ^{14}C residue [32]. Leptophos (*O*-4-bromo-2,5-dichlorophenyl *O*-methyl phenylphosphonothioate), another delayed neurotoxic chemical, was readily metabolized and excreted as water-soluble metabolites in the urine of rat [33,34] and mice [35], while in the hen only a small fraction is metabolized and excreted [36—38].

In summary, TOCP is readily absorbed from the GI tract and efficiently eliminated from the body of the rat, a species less sensitive to OPIDN. While in the hen [18,19], our previous study showed that TOCP and its metabolites were more persistant in the tissues, especially the target tissues for OPIDN (the sciatic nerve, spinal cord and the brain). Higher concentrations of TOCP and saligenin cyclic-*o*-tolyl phosphate were detected in the plasma and tissue of the hen relative to the rat at all time points. This may indicate that pharmacokinetics and metabolism may be responsible, in part, for species selectivity to OPIDN induced by TOCP.

Acknowledgements

The authors wish to thank Dr. Elizabeth Suwita for her help. We also thank Mrs. Mary Greene and Mrs. Eleanor Donham for their excellent secretarial help. This study was supported in part by NIEHS Grant No. ESO-3411, ESO-2721 and ESO-5154 and NIOSH Grants No. OHO-2003 and OHO-0823.

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