Toxicology Letters, 4 (1979) 21—24

DOES TRIPHENYL PHOSPHATE PRODUCE DELAYED NEUROTOXIC EFFECTS?

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(Received March 10th, 1979)
(Accepted March 14th, 1979)

SUMMARY

Previous reports of the delayed neurotoxicity of triphenyl phosphate in the cat have been re-evaluated in the light of discoveries of impurities in old samples and the non-neurotoxicity of the pure compound in hens. In a limited study using 99.99% pure synthetic triphenyl phosphate, cats given s.c. doses of 0.4 g/kg did not become ataxic; higher doses caused prostration some time after dosing but histological examination did not reveal any evidence of axon degeneration or demyelination in the spinal cord. There was no evidence that pure triphenyl phosphate causes delayed neurotoxic effects of the type produced by tri-*ortho*-cresyl phosphate.

INTRODUCTION

Some organophosphate esters can cause irreparable ataxia and paralysis in man, cat, hen and other species. 1—2 weeks after dosing some long axons of central and peripheral nerves begin to degenerate from the distal end. This delayed neurotoxic effect has often been referred to as demyelination [1] but myelin loss is secondary to axon degeneration and not easily seen in the early stages [2]. The primary biochemical event is inhibition of a nervous system enzyme known as neurotoxic esterase [3,4] and the process and its mechanism have been reviewed by Johnson [5].

Triphenyl phosphate is a potentially useful component in mixtures of phosphate esters prepared for use as hydraulic fluids and plasticisers. How-

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ever, the ester was classified as neurotoxic in the cat by Smith et al. [6]. They reported that 0.2 g/kg or above, injected subcutaneously, produced general muscular weakness and prostration. Toxic amounts given in one dose were said to result, after a period of time, in degeneration of nerve cells, whereas cumulation of the toxic dose during a period of several days was reported to result in degeneration of peripheral nerves [1]. It has since been shown that while some samples of triphenyl phosphate from coal-tar sources can be neurotoxic to hens, the pure compound does not produce this effect, even on repeated dosing, and does not inhibit hen brain neurotoxic esterase in vitro or in vivo [5,7,8]. It seemed worthwhile therefore to re-investigate the effects of this compound in cats, using a zone-purified sample supplied from a commercial source.

METHODS

Zone-refined triphenyl phosphate (declared purity 99.99%) was kindly supplied by FMC Corporation, Princeton, NJ. 5 cats were given triphenyl phosphate, with three others being held as controls. 2 cats received single doses of 0.4 g/kg of triphenyl phosphate dissolved in propylene glycol and injected beneath the skin behind one scapula. A control cat received an equivalent volume of propylene glycol injected into the same general area.

1 cat received a dose of 1.0 g/kg and 2 received doses of 0.7 g/kg of triphenyl phosphate, dissolved in corn oil, by injection beneath the skin of the back. 2 control cats received similar injections of corn oil.

The animals were observed for feeding, drinking and general behaviour. They were weighed at irregular intervals. Samples of blood from the 2 cats given 0.7 g/kg of triphenyl phosphate and from their 2 control animals were examined for cholinesterase activity in whole blood, plasma and red blood cells by a pH-stat method.

The cats were killed by i.v. overdoses of pentobarbital Na and were perfused with saline, to remove most of the blood, and then with 10% buffered formalin. Samples of brain stem and spinal cord were taken from all cats. Complete necropsies were carried out on the 2 cats given 0.7 g/kg of triphenyl phosphate.

RESULTS

The cats given triphenyl phosphate stopped eating and drinking at variable times after the doses. In general, larger doses caused earlier cessation of ingestion. All cats given triphenyl phosphate except one of those that received 0.4 g/kg lost weight. The 3 cats injected with propylene glycol, either alone or as the solvent for triphenyl phosphate, developed ulcers at the site of the injection. The presence of triphenyl phosphate in the propylene glycol did not seem to influence either the production or the healing of the ulcers in two of these cats.

The cat that lost weight after receiving 0.4 g/kg of triphenyl phosphate (about 31% of its original body weight, without any sign of unusual weakness or ataxia, during the 5 weeks after the dose) returned to about its original weight within 3 months after the dose and seemed then to be normal in both behaviour and appearance. The second of these cats never lost weight. These animals were not subjected to necropsy.

The cat given 1.0 g/kg of triphenyl phosphate was first noted to be anorexic 1 week after the injection. After 2 more weeks, the cat was unable to rise from the floor of its cage. On the following day, it had slow, laboured respiration, was nearly completely insensitive to external events and was judged to be moribund. By that time, it had lost almost 48% of its original weight. Sections of this cat's brain stem and spinal cord, stained with haematoxylin and eosin, showed normal nerve cell bodies in the gray matter; sections stained with luxol fast blue and cresyl violet did not reveal any evidence of axon degeneration or demyelination of axons in any tract.

The 2 cats given 0.7 g/kg of triphenyl phosphate both became anorexic soon after the injections. 1 cat went downhill more rapidly than the other, suffering from a persistent, brownish, watery diarrhoea and becoming prostrated within 3 days. On necropsy, 5 days after the injection, by which time this cat had lost 26.1% of its original body weight, two ulcers, from which fairly profuse bleeding had evidently occurred, were found near the pylorus of the stomach. The intestines were empty except for some brownish fluid. The mucosa of the small intestine was stained brownish to yellowish, the deeper stain being nearer to the stomach. The interior of the colon had a purplish red colour.

The second of these cats did not develop a watery diarrhoea similar to that seen in the first cat, but by the 7th day after the injection had lost 25% of its original body weight. It was prostrated, but was still capable of moving its legs feebly when stimulated. Upon necropsy, no ulcers were seen within this animal's gastrointestinal tract. The mucosa of the tract, with the exception of that of the oesophagus, was, however, markedly hyperaemic.

Histologic examination of sections of various organs of these two cats revealed generalised vascular damage, with perivascular oedema in many tissues. Oedema was especially evident in the colons, which had been denuded of their epithelial linings. The livers displayed fatty change and dilatation of the sinusoids. The kidneys seemed to be essentially normal in structure.

Sections of 11 levels of the nervous system, from cerebral cortex to peripheral nerve, dyed with haematoxylin and eosin, luxol fast blue and periodic acid-Schiff, Nissl, and Holmes stains did not provide any evidence of axon degeneration, demyelination or any other pathological change.

Samples of whole blood, plasma, and red blood cells taken from these two cats at necropsy contained cholinesterase activities that were within the same ranges as those of corresponding samples from the two control cats.

The doses of pure triphenyl phosphate which we here report to cause prostration, and which may lead to death, are two to three times those

reported by Smith et al. [6] to produce similar effects. We propose, with respect to their observation, that the sample of triphenyl phosphate may have contained impurities that were capable of producing axon degeneration and demyelination in the cat in the same manner as in the hen [6]. We believe also that they may have confused prostration, lassitude, weakness resulting from loss of blood or fluid and other toxic effects not related to the central nervous system, such as we saw and found in our cats at higher doses, with the actual paralysis that follows axonal damage and demyelination by truly neurotoxic organophosphorus compounds.

Triphenyl phosphate can be prepared from easily available synthetic phenol. It is highly unlikely that such material will contain the impurities which are responsible for the neurotoxicity of samples prepared from coal-tar sources. This should be recognised in the toxicological evaluation of plasticisers or other materials containing triphenyl phosphate.

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