Higher Chlorinated Dioxins Implicated in the Mortality of Young Pigs Kept on a Pentachlorophenol- treated Wooden Floor

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SUMMARY

Young pigs raised on a pentachlorophenol-treated wooden floor showed a high mortality. The deaths ceased when the original treated wood was covered with untreated plywood. Analysis of the wood, mother's milk, and young pig tissues was carried out for pentachlorophenol and chlorinated dibenzo-*p*-dioxins. Pentachlorophenol was found in the wood and mother's milk but not in the young pig tissues. Concentrations of the higher chlorinated dioxins were found in the wood at the ppm level, in the mother's milk at the low ppt level and in the skin and liver of the young pigs at the ppb level. A comparison of the concentrations of hexa-and hepta-dioxin isomers in the wood, and in the piglet skin and liver indicated that a selective absorption and/or metabolism of these isomers had occurred. The results of this case history implicate the higher chlorinated dioxins in the mortality of the young pigs and underline the danger of using technical pentachlorophenol for wood treatment in association with food producing animals.

RÉSUMÉ

L'implication de dioxines hautement saturées en chlore, dans la mortalité de porcelets gardés sur un plancher de bois traité au pentachlorophénol

Des porcelets gardés sur un plancher de bois traité au pentachlorophénol affichèrent un taux élevé de mortalité. Ils cessèrent toutefois de mourir, dès qu'on eut recouvert le plancher avec du contre-plaqué intact. On effectua une analyse du bois, du lait d'une truie et de certains tissus des porcelets, dans le but de démontrer la présence de pentachlorophénol et de dibenzo-*p*-dioxines chlorées. Le bois et le lait de la truie recelaient du pentachlorophénol, contrairement aux tissus des porcelets. On décela aussi de fortes concentrations de dioxines hautement saturées en chlore dans le bois et de faibles concentrations dans le lait de la truie; la peau et le foie des porcelets en contenaient cependant davantage. Une comparaison de la concentration des isomères des hexa-et des hepta-dioxines, dans le bois ainsi que dans la peau et le foie des porcelets, révéla qu'une absorption et/ou un métabolisme sélectifs de ces isomères avaient eu lieu. Les résultats de cette étude impliquent les dioxines hautement saturées en chlore dans la mortalité des porcelets et soulignent le danger inhérent à l'utilisation du pentachlorophénol pour le traitement du bois avec lequel les animaux destinés à la production de viande sont susceptibles de venir en contact.

INTRODUCTION

Poisoning of food producing, domestic and experimental animals can occur when they are exposed to contaminated bedding or wood. Pentachlorophenol (PCP) (1-8) is often implicated as the causative agent since it is a widely used wood preservative and has been detected in animal bedding. Occasionally dieldrin has been found to be the toxic contaminant (9-10). Recently, a more insidious type of toxicosis has been uncovered (11-12) in farm animals raised in contact with bedding or wood which contained chlorinated dibenzo-*p*-dioxins (dioxins) and chlorinated dibenzofurans (furans). Chickens raised on wood shavings containing dioxins and furans have shown an increase in the occurrence of liver fibrosis and morbidity. The livers contained readily measureable amounts of higher chlorinated dioxins (11). Michigan dairy cattle raised in contact with contaminated wooden buildings had reproductive problems and low milk yields (8). Their tissues contained low parts per billion (ppb) concentrations of the higher chlorinated dioxins (12). The presence of contaminants in the tissues of food producing animals is particularly disturbing since the residues can readily be passed on to humans. The purpose of this paper is to describe analytical results of a case history implicating higher chlorinated dioxins as the causative toxic agents in the death of piglets exposed to a recently PCP-treated wooden floor.

History

In the spring of 1979 piglets born in farrowing pens having a wooden floor vomited, showed locomotor problems (hopping gait), with some deaths occurring within 24-48 h. Postmortem examination failed to show the cause of death although there was a hyperkeratosis of the skin with a few focal points of necrosis. The sow did not exhibit any clinical signs of toxicity. The wood on the floor had been freshly treated with technical PCP. The mortality ceased when the original wood on the floor in the farrowing pens was recovered with untreated plywood.

Samples of the treated wood, sow's milk, and tissues from two piglets (liver, brain, kidney, serum, stomach content (piglet 1 only), and skin (piglet 1 only) were collected. The wood, milk, livers and the single stomach contents were analyzed for PCP by the Ontario Ministry of Agriculture and Food's Veterinary Services Laboratory, Guelph, Ontario. The method was based on gas chromatography (GC) with electron capture (EC) detection of the acetate derivative (13). Some samples were sent also to the Health Protection Branch laborato-

ries in Ottawa for determination of the dioxin content, a contaminant often associated with PCP. The tissue analysis consisted of extraction with chloroform-methanol, lipid degradation and removal with concentrated sulfuric acid, and chromatography of the extract on a Florisil column. Tissue sample extracts were purified further by separation into their dioxin congener groups by high performance liquid chromatography. Isomer separation was effected by a fused silica GC column connected directly to a mass spectrometry (MS) with selected ion monitoring for determination. Values quoted are uncorrected for recovery which average 60 to 80% in the 20 to 500 part per trillion (ppt) range depending on sample matrix and dioxin concentration.

Findings

The PCP concentrations in some of the samples are shown in Table I. As expected the treated wood had high amounts of PCP. The milk of the sow had a readily detectable but not abnormally high concentration of PCP. None could be found in the two livers or stomach contents of one of the piglets. Insufficient tissue was available to analyze the skin of the young pigs for PCP.

The dioxin concentrations in some of the samples are listed in Table II. The wood values are quoted in units of ppb and the rest of the samples in ppt (three orders of magnitude difference). The PCP used in this wood treatment contained the higher chlorinated dibenzo-*p*-dioxins (hexa, hepta, octa) in amounts typical of the technical product (16). Low or nondetectable amounts of the higher chlorinated dioxins were found in the sow's milk. Very high concentrations of octa and hepta dioxins were found in the one piglet skin available and the two livers. Little or no dioxins were detected in either the kidney or brain tissues or the serums. Tetrachlorodibenzo-*p*-dioxins (TCDD), particularly the higher toxic 2,3,7,8-TCDD isomer, were not found in any of these samples at the stated detection limit. The concentrations of the hexachlorodioxins in the tissues are not high when compared to the concentration of the less toxic octachlorodioxin or to previous reports (11,12,17,18) on higher chlorinated dioxins in tissues of food producing animals. The distribution of the ten possible hexa-isomers and the two possible heptachlorinated dioxin isomers in the wood and piglet 1 skin and liver are summarized in Table III. Figure 1 shows the chemical structure of 1,2,3,6,7,8-hexachloro-dibenzo-*p*-dioxin, one of the more toxic (17,18) hexa isomers. There were four hexa- and two hepta-isomers detected in the wood, three and two detected respectively in the skin and one of each in the liver. The isomeric ratio of the hexa- and hepta-dioxins also varied among the three samples with wood and skin having a different percentage than liver which contained 100% of a single isomer of both hexa-and hepta-dioxin.

TABLE I
PENTACHLOROPHENOL (PCP) RESIDUES[a] IN SELECTED SAMPLES FROM PIGS EXPOSED TO TREATED WOODEN FLOOR

Sample	PCP level in μg/g (ppm)
Wood	3100
Sow's milk	0.31
Young pig 1, liver	ND[b] (0.02)
Young pig 2, liver	ND (0.02)
Young pig 1 stomach content	ND (0.02)

[a]Samples analyzed by Veterinary Services Laboratory, Ontario Ministry of Agriculture and Food
[b]Not detected followed by the bracketed detection limit in ppm

TABLE II
DIOXIN LEVELS IN SAMPLES FROM PIGS EXPOSED TO PCP TREATED WOODEN FLOOR

Sample	Dioxin Level			
	Tetra	Hexa	Hepta	Octa
		ng/g (ppb)		
Wood	ND[a] (0.04)	10.8	157	1390
		pg/g (ppt)		
Sow's milk	ND[b] (10)	ND (15)	45 (1)	18
		Piglet tissue, pg/g (ppt)		
Skin 1	ND[b] (10)	52	790	3860[c]
Liver 1	ND (20)	71	1670	4800[c]
Liver 2	ND (10)	70	900	4200[c]
Kidney 1	ND (5)	32	-	27
Kidney 2	ND (5)	44	-	102
Brain 1	ND (20)	60	-	ND (10)
Brain 2	ND (20)	38	-	ND (10)
Serum 1	-	ND (10)	ND (10)	ND (10)
Serum 2	-	ND (10)	ND (10)	ND (10)

[a]ND — not detected followed by bracketed detection limit in ng/g
[b]Detection limit in ppt (pg/g)
[c]Average of two individual analyzes

DISCUSSION

Pentachlorophenol was not found in most of the piglet tissues even though high concentrations were present in the treated wood. Since purified PCP is only mildly toxic (15) to young pigs, it was not implicated in the mortality

TABLE III
DISTRIBUTION OF ISOMERS OF HEXA- AND HEPTA-CHLORINATED DIOXINS IN WOOD AND PIGLET 1 SKIN AND LIVER

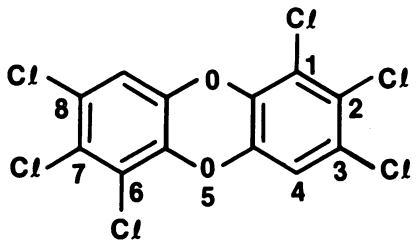
Sample	Wood		Skin		Liver	
Isomer[a]			Hexa			
	ppb[b]	%	ppt[c]	%	ppt[c]	%
1,2,4,6,7,9-	0.2	2	2	4	-	-
1,2,3,6,7,9-	5.1	47	8	16	-	-
1,2,3,6,7,8-	4.9	45	42	80	71	100
1,2,3,4,6,8-	0.6	6	-	-	-	-
Total	10.8	100	52	100	71	100
			Hepta			
Isomer[a]						
1,2,3,4,6,7,9-	56	36	126	16	-	-
1,2,3,4,6,7,8-	101	64	664	84	1670	100
	157	100	790	100	1670	100

[a]Elution order on GC OV-17 column; for numbering, see Figure 1
[b]Parts per billion (ng/g)
[c]Parts per trillion (pg/g)

seen in this instance. The tetrachlorinated dioxins were not suspected in this case because none were detected and 2,3,7,8-TCDD has never been reported in association with PCP (16).

The wood sample contained significant concentrations (ppb) of the higher chlorinated dioxins. The ratio of octachlorodioxin to PCP (about 500 parts per million) in the wood sample is typical of technical PCP currently being marketed as a wood preservative in Canada (16). The piglet skin and livers contained high tissue concentrations of the higher chlorinated dioxins even though the relative amounts of hexa-, hepta-, and octa between wood and tissues did not vary markedly (hexa was 0.5 to 1.5% of octa and hepta was 10 to 35% of octa in the three samples). Since the tissue concentrations are in the ppb area, they appear to be the cause of the mortalities.

The route of dioxin exposure does not appear to be the sow's milk as it contained little or no dioxins. However, the young pig skin contained high concentrations of dioxins indicating a dermal route of exposure. The two serum samples contained no measureable dioxins at the stated detection limits. In view of the presence of high amounts in both the skin and liver tissues, the absence of detectable amounts in the serum is surprising. Only one isomer of the hexa-group (1,2,3,6,7,8-,), (Figure 1), was found in the liver, kidney and brain. One of the two hepta-isomers (1,2,3,4,6,7,8-) was detected in the liver. The number of hexa- and hepta-isomers in the skin was intermediate to that detected in the wood and liver indicating a selective absorption and/or metabolism was present from the wood to the skin and into the tissues. Such a selective absorption has been noted previously with dioxins in chickens (17) and dairy cows (18). Those dioxins substituted with chlorine in the four lateral positions are absorbed and retained; others possessing different chlorine patterns and decreased toxic effects are either not absorbed or rapidly metabolized.



1, 2, 3, 6, 7, 8 — HEXACHLORODIBENZO-P-DIOXIN

FIGURE 1. Chemical structure of 1,2,3,6,7,8-hexachlorodibenzo-p-dioxin illustrating the dioxin ring system and the numbering of the positions where from one to eight chlorine (Cl) atoms can be substituted.

Data on toxic concentrations of dioxins in pig tissues are not available. The low ppb levels of octa and hepta dioxins found in these pig samples can be compared to the low ppb values found in dairy cow and calf liver and fat (12) which caused clinical toxic signs but no acute fatal effects. In the present case, only newborn animals suffered fatalities. Their metabolic systems (enzymes, detoxification mechanisms) had probably not yet developed to handle the acute exposure, an observation noted previously (1) indicating that the piglets were more susceptible to poisoning than older animals.

Most of the technical PCP used as a wood preservative contains significant levels of the higher chlorinated dioxins. Because of this fact, more stringent regulations have been enacted by Agriculture Canada concerning the use of PCP near farm animals although no control is exercised over the quality and purity of the PCP itself. The concentrations of dioxins in the young pig livers and the accumulation of the more toxic types in the tissues are strongly suggestive in this case history that dioxins were responsible for the mortality. The cessation of losses after the treated wood was covered adds further support to the diagnosis of dioxin toxicity. The investigation underlines the danger of technical PCP use near food producing animals, both to the animals and to humans who may consume contaminated food products. The dioxins detected in this report are not as toxic as 2,3,7,8-TCDD but do have potent biological properties.

ACKNOWLEDGMENTS

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ERRATUM

Bovine cryptosporidiosis: Clinical and pathological findings in forty-two scouring neonatal calves

Can. vet. J. 23: 343-347 (December 1982)

Because of poor reproduction and indiscriminate cropping Figures 2 and 5 in the above named paper were less than satisfactory. We are repeating the two figures and apologize to the authors and the readers for any inconvenience caused by our errors.

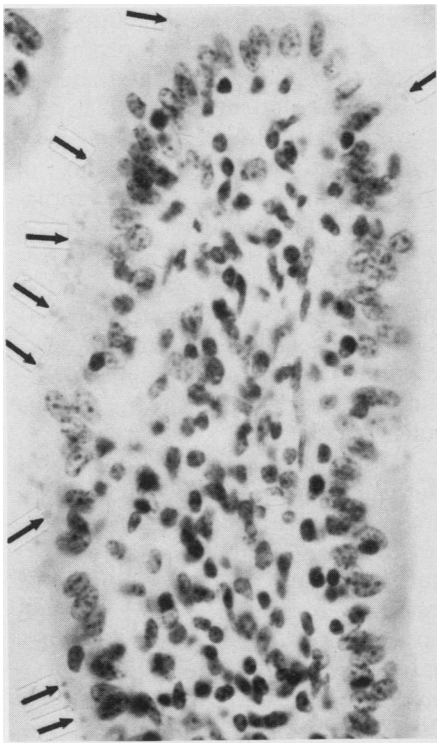

FIGURE 2. *Cryptosporidia* organisms (arrows) embedded in the brush border of a jejunal villus of a three week old calf. Moderate numbers of mononuclear cells have invaded the lamina propria. H & E. X125.

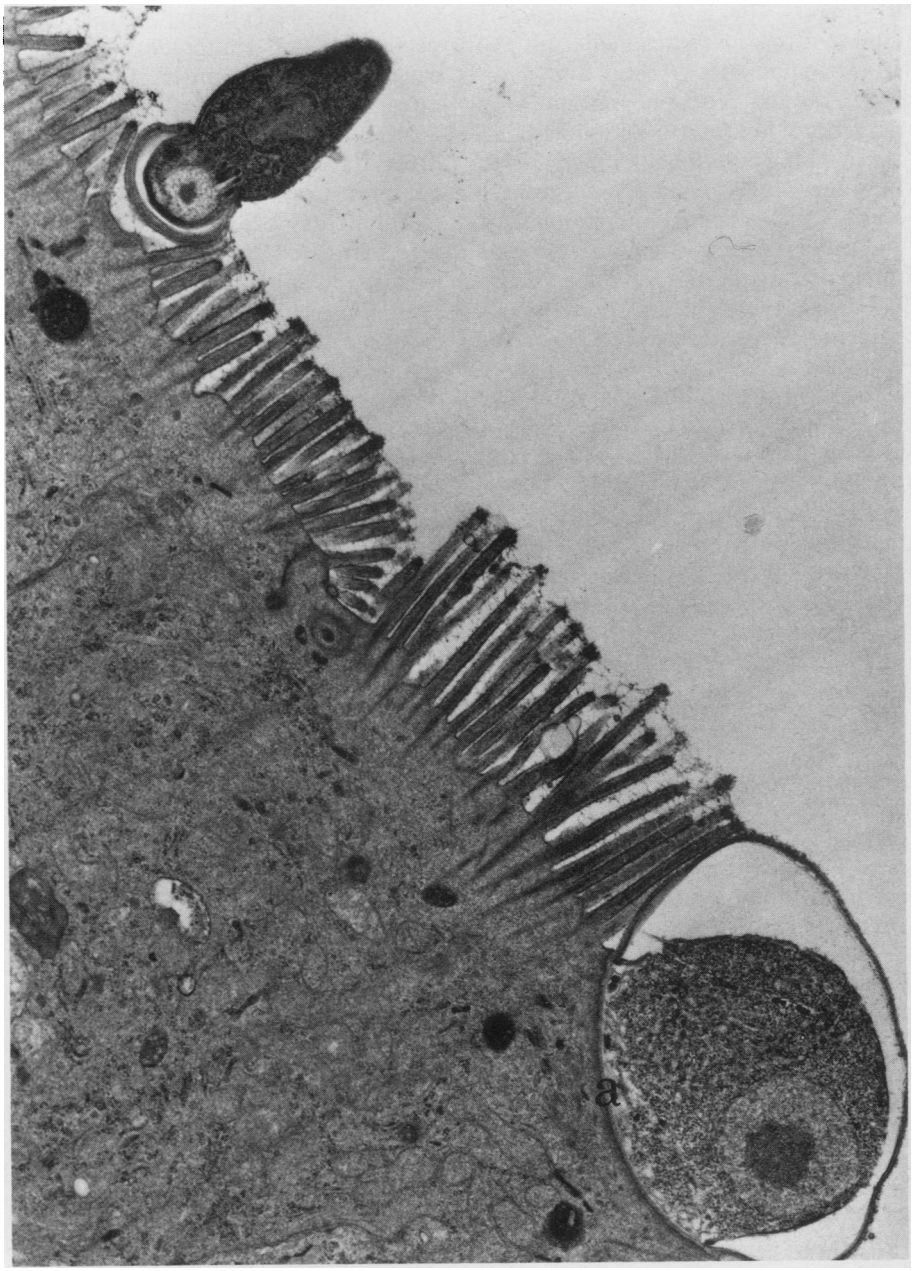

FIGURE 5. Ileal enterocytes with trophozoite (below) and merozoite (above). Merozoite is apparently embedding into or about to be enveloped by a microvillus. a = electron dense attachment zone. TEM. X14 465.