シアノフェンホス(シュアサイド(R))連続投与による乳牛のミルクおよび肉中における残留について

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著者	宮本, 純之 Keplinger, M.L. Wingender, R.J. 滝本, 善之 Jenkins, D.H.
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Original Articles

Residue Study with Cyanofenphos in Milk and Meat Following the Subacute Feeding to Dairy Cattle

Junshi MIYAMOTO, Moreno L. KEPLINGER,* Ronald J. WINGENDER,*
Yoshiyuki TAKIMOTO and Donald H. JENKINS*

Research Department, Pesticides Division, Sumitomo Chemical Co., Ltd., Takarazuka, Hyogo 665, Japan
**Industrial BIO-TEST Laboratories, Inc., Illinois, U.S.A.*

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Four groups of 4 dairy cows were fed a diet containing 0, 5, 15 and 50 ppm cyanofenphos, *O*–ethyl *O*–4–cyanophenyl phenylphosphonothioate (Surecide®) for 30 days. Three cows from each group were sacrificed on day 30 and the remainders were kept on a normal diet for a 30-day recovery period. Milk samples were taken at specified intervals during the entire experimental period, and liver, kidney, muscle and fat samples were collected at sacrifices. Cyanofenphos and cyanofenphos-oxon residues in milk and tissues were extracted with methanol/acetonitrile and determined by gas chromatography on a 3% OV-1 column using a flame photometric detector.

In the milk of cows the residues were found only on the 50 ppm treatment. The highest level of cyanofenphos (0.25 ppm) occurred at day 2 and 4. The oxon level (0.06 ppm) peaked at day 4. The concentration of both compounds in milk decreased thereafter, and after 30 days of feeding the residue levels of cyanofenphos and cyanofenphos-oxon were less than the detection limits. The 50 ppm treatment for 30 days resulted in residues of 0.9 ppm cyanofenphos and 0.09 ppm cyanofenphos-oxon on an average in the fat, whereas other tissues generally contained one tenth the amount of these compounds.

At the lower dosages any detectable amounts of cyanofenphos or its oxon were hardly observed. At day 60, trace amounts (0.005 ppm) of cyanofenphos were sporadically detected in the tissues.

INTRODUCTION

Cyanofenphos, Surecide® or *O*–ethyl *O*–4–cyanophenyl phenylphosphonothioate is an organophosphorus insecticide effective against *Lepidoptera, Diptera, Orthoptera, Hemiptera* and *Coleoptera,* and is widely used for the control of various plant pests and livestock insects. Extensive residue study in crops carried out mainly in Japan[1] reveals that the residue figures of the compound in major crops are generally favorable for recommending its use for agricultural purposes, however that the residue level is significantly higher in dry rice straw than are those of other comparable organophosphorus compounds such as Cyanox® [*O*,*O*–dimethyl *O*–(4–cyanophenyl)phosphorothioate] and Sumithion® [*O*,*O*–dimethyl *O*–(3–methyl–4–nitrophenyl) phosphorothioate],[2-4] being around 5 ppm at its maximum. Since rice straw is often used as a feed for cattle in Japan, it is necessary to ascertain from the viewpoint of public health whether feeding of cyanofenphos results in a measurable amount of the residues of the compound and the toxic metabolites therefrom in milk and meat of cows or not. Accordingly, cyanofenphos was administered consecutively to dairy cows, and

milk and edible tissues were analyzed for the residue of cyanofenphos and cyanofenphos-oxon.

MATERIALS AND METHODS

1. *Special Chemicals*

Cyanofenphos used for the feeding study was a technical product of Sumitomo Chemical Co., Ltd., lot No. E199 with the purity of 92%. Purified cyanofenphos for analytical standard was prepared by repeated recrystallization of the technical product from ethanol, melting at 83°C. Cyanofenphos-oxon or *O*–ethyl *O*–4–cyanophenyl phenylphosphonate, $n_D^{27°}=1.5512$, was synthesized by reaction of *O*–ethyl phenylphosphonochloridate with 4–cyanophenol in the presence of triethylamine at 20°C, and purified by silica gel column chromatography with a mixture of *n*–hexane and acetone (9:1).

2. *Feeding Study*

Sixteen healthy, mature Holstein dairy cows at various stages of lactation were used in the investigations. All animals were subjected to 15–day pretest period which permitted adjustments in feeding, compilations of daily milk records and observations of behavioral reactions. Each animal was given a total daily ration of 18 kg, consisting of 4.5 kg of commercial dairy feed (Purina Dairy Chow, Ralston Purina Company, St. Louis) and the balance of clover-prairie hay. Water was permitted at all times.

Four cows were selected for each of 4 groups on the basis of the average milk weight during the pretest period and they were given 4.5 kg of the dairy chow containing 0, 20, 60 and 200 ppm of technical cyanofenphos (or 0, 5, 15 and 50 ppm based on a total dairy food consumption of 18 kg/cow) and 13.5 kg of clover-prairie hay. Daily milk weight for each cow was recorded. A one liter aliquot of milk was retained from each cow on test days 0, 2, 4, 8, 14, 18, 22, 26 and 30 and frozen until analyzed. On test day 30, 3 cows from each group were sacrificed and examined for gross pathologic changes. Samples of about 500 g of liver, kidney, muscle (front leg, rear leg and loin, combined) and fat (kidney and subcutaneous, combined) were collected from each animal and quickly frozen at −18°C until analyzed (for 4 months at the longest).

The remaining animal from each group was placed on a 30-day recovery period, receiving 4.5 kg basal ration and 13.5 kg clover-prairie hay during the period. On day 37, 44,51 and 60, a one liter milk sample was collected from each cow. On day 60, the remaining animals were sacrificed and sampled in the same manner as those sacrificed on day 30.

All animals were observed through the investigation for signs of toxicity and untoward behavioral reactions.

3. *Analytical Procedures*

Cyanofenphos and cyanofenphos-oxon in milk and tissue samples were extracted and cleaned up in essentially the same manner as that reported elsewhere.[5] The final extract was subjected further to a Florisil column chromatographic clean-up as in the following: A chromatographic column of 22 mm in i.d. was prepared with 10 g of Florisil (60–100 mesh) deactivated with 10% of an aqueous buffer (0.01 M NaH_2PO_4 adjusted to pH 7 with NaOH) and topped with *ca.* 1 cm of anhydrous sodium sulfate. The column was wet with *n*–hexane and the residue transferred to the column using portions of *n*–hexane. Both the cyanofenphos and its oxon were eluted from the column using 150 ml of a mixture of methylene chloride: acetonitrile: *n*–hexane (50:15:100). The eluate was collected and evaporated to near dryness. The residue was quantitatively transferred to a 15 ml graduated centrifuge tube with *n*–hexane and the volume adjusted for quantitation by gas chromatography.

The gas chromatographic determination was carried out with a Tracor MT–220 gas chromatograph equipped with a flame photometric detector in the phosphorus mode under the operational conditions; Column, 75 cm × 3 mm i.d. glass packed with 3% OV-1 on Chromosorb W 80/100 mesh; carrier, nitrogen at 65 ml/min; temperature of column, detector and inlet, at 185°C, 225°C and 200°C, respectively. Under these conditions retention times were 3–1/2 and 2–1/2 min for cyanofenphos and cyanofenphos-oxon, respectively. To eliminate tailing, it was necessary to use a glass insert packed with glass wool prior to the chromatographic column. Both column and packed

insert were treated with a silylating agent (Silyl–8, Pierce Chemical Co., Rockford, Illinois). Due to the large amount of coextractives, the first 1 cm of packing in the column and the glass wool were replaced after about 30 sample injections during analysis.

Samples of control milk and tissues were fortified with cyanofenphos and cyanofenphos-oxon at various levels (0.005–1 ppm) to determine recovery. The recovery averages 95% and 103% in milk and 92% and 100% in tissues for cyanofenphos and cyanofenphos-oxon, respectively.

RESULTS

There were no adverse reactions due to the feeding of cyanofenphos in the following items; milk production, food consumption, mortality and behavioral reactions, and gross pathologic findings in tissues at sacrifice.

Residues of cyanofenphos and cyanofenphos-oxon in milk were found only from cows given a diet containing 50 ppm of the compound (Table 1). The highest levels of cyanofenphos were found in the test day 2 and test day 4 samples in this test group, amounting approximately to 0.25 ppm. Cyanofenphos-oxon levels peaked on test day 4 (0.06 ppm). Thereafter, the residues tend to decrease, although the animals were still kept on the diet containing cyanofenphos. Residue levels of cyanofenphos and its oxon in the recovery samples collected on days 37, 44, 51 and 60 were below the limit of detection of the method (<0.002 ppm for cyanofenphos and <0.004 ppm for cyanofenphos-oxon in 50 g samples).

Residue levels of both compounds were highest in the fat samples and decreased in the order fat≫ liver> kidney≥ muscle in the tissues, as shown in Table 2. In the tissues of the remaining animals kept on the basal diet for another 30 days (recovery), the residue contents decreased and 0.005 ppm of cyanofenphos was sporadically detected. No cyanofenphos-oxon was found in the recovery samples.

DISCUSSIONS

Consecutive feeding of 50 ppm cyanofenphos (10 times excess of the maximum residue in dry rice straw) to dairy cows results in a measurable amount of the residue in milk, as shown in Table 1. The highest concentration (*ca.* 0.25 ppm) occurs shortly after initiation of the feeding (test days 2 and 4), and thereafter the level tends to decrease, although the cows have been kept on the cyanofenphos containing diet. The tendency might be partly due to the enhanced metabolic rate in the animals, as suspected by other workers,[6] and such an acclimatization or tolerance is observed in the chronic feeding of cyanofenphos to rats as well, where blood cholinesterases inh bited at the early stages of the experiment

Table 1 Results of the analysis of milk samples for cyanofenphos and cyanofenphos-oxon.[a]

Dietary concentration of cyanofenphos (ppm)	Concentration in milk (ppm)								
	Day 0	Day 2	Day 4	Day 8	Day 14	Day 18	Day 22	Day 26	Day 30
	Cyanofenphos								
0	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002
5	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002
15	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002
50	<0.002	0.246 ±0.078	0.227 ±0.052	0.051 ±0.022	0.067 ±0.028	0.022 ±0.008	0.064 ±0.027	0.043 ±0.022	0.027 ±0.011
	Cyanofenphos-oxon								
0	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004
5	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004
15	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004
50	<0.004	0.020 ±0.002	0.061 ±0.003	0.019 ±0.005	0.028 ±0.008	0.012 ±0.002	0.032 ±0.014	0.015 ±0.004	0.011 ±0.003

[a] The results are expressed as the mean value for 4 cows ± standard error.

Table 2 Results of the analysis of tissue samples for cyanofenphos and cyanofenphos-oxon.

Dietary concentration of cyanofenphos (ppm)	Animal number	Concentration (ppm)			
		Fat	Liver	Kidney	Muscle
		Cyanofenphos			
0	1	<0.002	<0.002	<0.002	<0.002
	2*[a]	<0.002	<0.002	<0.002	<0.002
	3	<0.002	<0.002	<0.002	<0.002
	4	<0.002	<0.002	<0.002	<0.002
5	5	<0.002	<0.002	<0.002	<0.002
	6*	0.005	<0.002	<0.002	<0.002
	7	0.011	<0.002	<0.002	<0.002
	8	<0.002	<0.002	<0.002	<0.002
15	9	0.013	0.006	<0.002	<0.002
	10*	<0.002	<0.002	<0.002	<0.002
	11	0.013	0.012	<0.002	<0.002
	12	0.012	0.004	0.005	<0.002
50	13	1.59	0.131	0.025	0.019
	14	0.121	0.033	<0.002	0.004
	15	0.975	0.125	0.011	0.006
	16*	0.005	<0.002	0.006	<0.002
		Cyanofenphos-oxon			
0	1	<0.004	<0.004	<0.004	<0.004
	2*	<0.004	<0.004	<0.004	<0.004
	3	<0.004	<0.004	<0.004	<0.004
	4	<0.004	<0.004	<0.004	<0.004
5	5	<0.004	<0.004	<0.004	<0.004
	6*	<0.004	<0.004	<0.004	<0.004
	7	<0.004	<0.004	<0.004	<0.004
	8	<0.004	<0.004	<0.004	<0.004
15	9	0.013	<0.004	<0.004	<0.004
	10*	<0.004	<0.004	<0.004	<0.004
	11	0.006	0.007	<0.004	<0.004
	12	<0.004	<0.004	0.009	<0.004
50	13	0.126	0.103	<0.004	0.008
	14	0.044	0.006	<0.004	<0.004
	15	0.085	<0.004	<0.004	0.005
	16*	<0.004	<0.004	<0.004	<0.004

[a] The animals marked with * were sacrificed on test day 60.

gradually recover during the subsequent feeding.[7]

Cyanofenphos-oxon is apparently formed in cows just like in mice,[8] as the oxygen analog is also detected in the milk samples (Table 1). The maximum level peaks on test day 4 (0.06 ppm), too.

The total residue of cyanofenphos and cyanofenphos-oxon at its maximum is 0.288 ppm (test day 4), and the ratio of the residue level to dietary concentration of the compound (0.288/50=0.0058) is much lower than that of total BHC in cow milk surveyed in Japan several years ago[9] (ratio of total BHC residue in fresh milk to total BHC content in rice straw, 0.23–0.39). The difference between total cyanofenphos and total BHC is even more marked, if taken into account of the average content of cyanofenphos and its oxon in milk samples during 30-day feeding period (0.119 ppm instead of 0.288 ppm). Once cyanofenphos is removed from the diet, no residues of the parent compound and its oxon are detected in milk.

Among 4 tissues analyzed, fat contains highest residue of cyanofenphos and cyano-

fenphos-oxon, followed by liver, kidney and muscle (Table 2). Only in a fat sample from cows fed 50 ppm of cyanofenphos, the residue of cyanofenphos above 1 ppm is found. The residues of both cyanofenphos and cyanofenphos-oxon have decreased and have been hardly detected, when the animals were fed the diet free of cyanofenphos.

By feeding of 15 ppm and 5 ppm cyanofenphos cow milk contains neither cyanofenphos nor cyanofenphos-oxon. In fat and liver a trace amount of the residues (less than 0.01 ppm) is sporadically detected.

Based on the above findings, cyanofenphos is likely to be rapidly metabolized in cows and not to accumulate in the tissues, and it seldom occurs that feeding of cyanofenphos containing rice straw to dairy cows results in a measurable amount of the residues of cyanofenphos and its oxon in fresh milk and tissues.

要　　約

シアノフェンホス（シュアサイド®）連続投与による乳牛のミルクおよび肉中における残留について宮本純之，M.L. Keplinger, R.J. Wingender, 滝本善之，D. H. Jenkins（住友化学工業株式会社，Industrial BIO-TEST Laboratories）.

シアノフェンホス（シュアサイド）*O*–エチル *O*–4–シアノフェニルフェニルホスホノチオエートを 0, 5, 15, 50 ppm の割合で含んだ飼料で各群4頭の乳牛を30日間飼育し，ミルクを採取するとともに3頭を30日目に解剖して肝臓，腎臓，筋肉および脂肪を得た．各群残り1頭はさらに30日間正常飼料で飼育しミルクを採取したのち屠殺した．ミルク，組織中におけるシアノフェンホスおよびシアノフェンホスオキソンをメタノール・アセトニトリル混液で抽出後 FPD 付きガスクロマトグラフで定量したところ，50 ppm 処理区のミルク中にのみシアノフェンホスが認められ処理開始2日および4日後で最高 0.25 ppm に達した．オキソン体も同じく4日後に最高の 0.06 ppm に達したが，両化合物ともその後減少し，30日後では検出限界以下となった．50 ppm 区30日後における脂肪中のシアノフェンホスおよびオキソン体の残留量はそれぞれ平均 0.9 ppm および 0.09 ppm であり，他の組織中ではこれらの残留量はその約 $^1/_{10}$ であった．15, 5 ppm 処理区ではミルク中の残留量はすべて検出限界以下，組織中にもほとんど残留を認めなかった．

60日後では各処理区を通じて少量 (0.005 ppm) のシアノフェンホスが一部の組織中に検出されたにすぎず，オキソン体はまったく存在しなかった．

REFERENCES

1) Anonymous: Technical Report of Sumitomo Chemical Co., Ltd., submitted to FAO (1975)
2) Y. Takimoto & J. Miyamoto: Unpublished observation
3) Y. Takimoto & J. Miyamoto: "Residue Reviews," ed. by F. A. Gunther, Springer Verlag, New York, Vol. LX, p. 83, 1976
4) Anonymous: "1974 Evaluations of Some Pesticide Residues in Food," FAO, Rome, p. 335, 1975
5) J. Miyamoto, Y. Sato & S. Suzuki: *Botyu-Kagaku* **32**, 95 (1967)
6) Y. Misu, T. Segawa, I. Kuruma, M. Kojima & H. Takagi: *Toxicol. Appl. Pharmacol.* **9**, 17 (1966)
7) T. Kadota, S. Hosokawa, H. Kohda, S. Sugiura & J. Miyamoto: Speech at 3rd Meeting of Toxicological Association (Japan) (1974)
8) S. Kato & I. Yamamoto: Private communication
9) H. Tanabe: "Environmental Toxicology of Pesticides," ed. by F. Matsumura, G. M. Boush and T. Misato, Academic Press, New York, p. 242, 1972