A Toxicological Study of Di-isopropylnaphthalenes (KMC) and 1-Phenyl-1-xylyl-ethanes (SAS)

I. Distribution and Disappearance of KMC and SAS in Rats

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Abstract: Di-isopropylnaphthalenes (KMC) and 1-phenyl-1-xylyl-ethanes (SAS) have been widely used, especially in non-carbon paper (pressure sensitive copying paper), since 1971 in place of PCBs. The toxicity of the solvents has been studied as one of the national special studies in Japan. First, in 1972, distribution and disappearance in the living body was investigated in rats. Although a little accumulation of the substances was observed in the fat after a single oral dose, it was later found that continuous oral administration for a month caused no accumulation in the other organs. Accumulation in the liver, blood, kidneys and brain was not observed. In experiments in vitro, furthermore, the substances were found to be decomposed by liver homogenate. The rates of decomposition of all components of KMC were similar, but the rates for SAS were not. The rate for 1-phenyl-1-orthoxylyl-ethane was larger than that for 1-phenyl-1-metaxylyl-ethane.

Keywords: Distribution—Decomposition—Accumulation—Metabolism—1-Phenyl-1-xylyl-ethanes (ASA)—Di-isopropylnaphthalenes (KMC)

INTRODUCTION

In February 1971 two classes of chemical substances, 1-phenyl-1-xylyl-ethanes (SAS) from Nippon Petrochemicals Co. Ltd. and isopropylnaphthalenes (KMC) from Kureha Chemical Industry Co. Ltd., were first used as solvents in micro-capsules for non-carbon paper (pressure sensitive copying paper). At the time, the toxicity of PCBs (polychlorinated biphenyls) on the living body was fairly well proved and the production of PCBs in Japan was stopped in 1972. Both SAS and KMC were developed as new solvents in place of PCBs. In 1972, the Science and Technology Agency of Japan designed a special study of the toxicity of PCBs and PCB replacements¹⁾. We studied the PCB replacements SAS and KMC.

The outline of this investigation was reported to the Science and Technology Agency of Japan in 1973.¹⁾

This paper describes the distribution and disappearance of SAS and KMC in organs of the rat. In our next papers, the toxicity of SAS and KMC observed in rats and the results of health examinations of SAS and KMC workers, which were carried out simultaneously with this experiment in 1972, will be reported¹⁾. Before our study in 1972, no experiment except those performed by investigators of the manufacturers or participating universities had been carried out^{2,3)}. Later Iwahara⁴⁾ showed that di-isopropylnaphthalenes have a short half-life in mice. Kojima *et al.*⁵⁾ reported a similar observation on mono- and di-isopropylnaphthalenes in rats. Sumino⁶⁾ reported contamination of estuarine mud and biota by di-isopropylnaphthalenes. Addison *et al.*⁷⁾ investigated the metabolism of SAS and KMC, and demonstrated induction of hepatic mixed function oxidase activity in trout. Although our experiments were performed in 1972, we will discuss the results taking into consideration the observations of many investigators.

MATERIALS AND METHODS

1. *Animals*

Male JCL-SD rats, 8 weeks old, were used. For the test for accumulation of SAS-296 or KMC-A, 0.1 g of the substance per kg of body weight was administered orally just once, or once a day for a month.

2. *SAS and KMC*

SAS contains two main isomers, 1-phenyl-1-orthoxylyl-ethane and 1-phenyl-1-metaxylyl-ethane. The main commercial preparations of SAS are SAS-295 and SAS-296 which contain the two isomers in different proportions. SAS-296 was used in this study.

Two species of KMC are used commercially as KMC-A and KMC-R. KMC-A consists of di-isopropylnaphthalenes and little other isopropylnaphthalenes. KMC-R has two main components, methyl-isopropylnaphthalenes and dimethyl-isopropylnaphthalenes. In this study, KMC-A was used.

SAS and KMC are not always used in industry in the composition described above; rather the composition varies according to the purpose of their use in various industrial products.

3. *Determination of SAS and KMC in tissues*

Tissues of rats administered SAS or KMC were homogenized by a polytron homogenizer. SAS or KMC in the homogenate was extracted with n-hexane, of which the purity was sufficient for PCB determination. The hexane solution was dehydrated by adding Na₂SO₄ powder, concentrated if necessary, and gaschromatographed with FID detector (Perkin Elmer 900).

These procedures were nearly the same as those for PCB determination. The conditions of gaschromatography were as follows: column, glass column of 1/4" ×

6 feet, 1.5% SE-30, Chromosorb W 60–80 mesh; carrier gas, N₂ 30 ml/min; temperature, column 160°C, injector 220°C, detector 190°C; sample, 1 μ l.

4. *Decomposition of SAS and KMC by rat liver homogenate*

Excised rat liver was washed three times with 1.15% KCl solution and a 25% homogenate in 1.15% KCl solution was prepared. The nuclear fraction was removed by centrifugation. SAS-296 and KMC-A as substrates were prepared by mixing a drop of SAS-296 or KMC-A with 100 ml of 1.15% KCl solution in a Buchner funnel. After standing overnight, the lower part was removed and used as substrate solution. The assay for SAS-296 or KMC-A decomposition was performed with the following reaction mixture: 2.5 ml of liver homogenate, 0.1 ml of 0.5 M phosphate buffer of pH 7.4, 0.4 ml of 1.15% KCl solution, 0.05 ml of MgCl₂ solution (final concentration 75 μ moles), 0.05 ml of nicotinamide (50 μ moles), 0.05 ml of fumarate (30 μ moles), 0.05 ml of glucose-6-phosphate (30 μ moles), 0.05 ml of NADP (0.15 μ moles), 0.05 ml of ATP (0.72 μ moles), 0.01 ml of glucose-6-phosphate dehydrogenase (Sigma) and 2.5 ml of substrate solution. The total volume of the reaction mixture was 5.81 ml. The decomposition rate was calculated from the amount of SAS or KMC decomposed in 20 minutes. The reaction was carried out at 37°C.

5. *Distribution of SAS-296 or KMC-A in rats*

One group of animals received 0.1 g of SAS-296 or KMC-A per kg of body weight orally, and were killed 0, 2, 4, 24 and 48 hours later. The amounts of SAS-296 or KMC-A in various organs were determined by gaschromatography.

Another group of animals received the same dose every day for a month, and the distribution in the body was determined at 2, 4 and 24 hours, and 7 and 30 days after the final dose.

RESULTS

1. *Gaschromatography of SAS-296 and KMC-A*

A gaschromatogram of SAS-296 is shown in Fig. 1. The two main peaks correspond to 1-phenyl-1-orthoxylyl-ethane and 1-phenyl-1-metaxylyl-ethane, respectively. Fig. 2 shows a gaschromatogram of KMC-A. The several peaks are diisopropyl-naphthalenes as ascertained by investigators in Kureha Chemical Industry Co. Ltd., the producer of KMC. The investigators in both plants also showed that the sensitivity of each component on the chromatogram was nearly the same, and that, therefore, the total amount of the components could be calculated as the sum of the peak areas. Determination of SAS or KMC in the body was performed according to the above results.

2. *Distribution and disappearance of SAS or KMC in the body*

In Figs. 3 and 4, the distribution and disappearance of SAS-296 and KMC-A

Fig. 1. Gaschromatogram of SAS-296.

during 48 hours after a single oral dose of 0.1 g per kg of body weight are shown. Both SAS and KMC were found in large quantities in body fat and in subcutaneous fat 2 hours after the dose. The substances in fat increased with time until 24 hours. Nearly the same amount was present in the liver as in fat at 2 hours, but the disappearance rates were fairly large, especially in SAS-296. The concentrations in the blood were smaller than those in the heart, the kidneys and the brain. From the results shown in the figures, the rate of disappearance of SAS-296 was deemed larger than that of KMC-A.

The composition of KMC in various organs during its disappearance did not change in comparison with that of the KMC-A administered. On the other hand, the composition of SAS in the organs changed in comparison with that of the

Fig. 2. Gaschromatogram of KMC-A

SAS-296 administered. At 2 hours after administration, the amount of 1-phenyl-1-metaxylyl-ethane was about three times larger than that of 1-phenyl-1-orthoxylyl-ethane in each organ. The change in SAS composition in the organs can be explained as the difference in rates of decomposition of the components in the liver as will be shown later.

The experiments shown in Figs. 5 and 6 were conducted to determine whether or not SAS and KMC accumulated in the body when they were given continuously for a month. The amounts of SAS or KMC in the organs were determined 2, 4, and 24 hours, and 7 and 30 days after the final dose. The amounts of SAS or KMC in the organs 2 hours after the final dose were of nearly the same order as those after a single dose showing that neither SAS nor KMC accumulates in the

Fig. 3. Distribution and disappearance of SAS-296 after a single oral dose of 0.1 g per kg of body weight (left).

Fig. 4. Distribution and disappearance of KMC-A after a single oral dose of 0.1 g per kg of body weight (right).

Fig. 5. Distribution and disappearance of SAS-296 after continuous administration of 0.1 g per kg of body weight every day for a month (left).

Fig. 6. Distribution and disappearance of KMC-A after continuous administration of 0.1 g per kg of body weight every day for a month (right).

organs. Although a little accumulation was observed in fat, its influence on the distribution and the amounts in other organs seemed to be negligible.

3. Decomposition of SAS-296 and KMC-A by liver homogenate

To the reaction mixture described above, liver homogenate from male rats and then SAS-296 or KMC-A of various concentrations were added at 37°C. Concentrations of the substrates at the beginning of the reaction and 2 hours later

Table 1. Decomposition of SAS-296 and KMC-A by liver homogenate at 37°C.

		Initial concentration ($\mu\text{g/ml}$)	Remaining concentration at 2 hrs. ($\mu\text{g/ml}$)	Decomposition rate (%)	
SAS-296		4.6	3.1	32	
		3.6	2.6	28	
		2.6	2.0	23	
	1-phenyl-1-metaxylyl-ethane
	2.0	0.8	60	
		1.6	0.7	58	
		1.1	0.4	58	
	1-phenyl-1-orthoxylyl-ethane				
KMC-A		a	1.6	0.8	50
		a	1.2	0.7	41
		b	0.75	0.4	48
		b	0.42	0.24	42
	di-isopropylnaphthalene				

Table 2. Michaelis' constant (K_M) and maximum reaction velocity (V_m) calculated from the results shown in Table 1.

		V_m		K_M	
		$\left(\frac{\mu\text{g/ml}}{\text{g}\cdot\text{hr}}\right)$	$\left(\frac{\mu\text{M/l}}{\text{g}\cdot\text{hr}}\right)$	$(\mu\text{g/ml})$	$(\mu\text{M/l})$
SAS-296		3.6	17	11.2	53
	10.0	50	16.2	77
KMC-A	a	3.3	16	5.7	27
	b	1.8	8.6	4.3	21

were determined by gaschromatography. The results are shown in Table 1. The rate of decomposition was dependent on the initial concentration added. In SAS-296, the rate of decomposition of one component, 1-phenyl-1-orthoxylyl-ethane, was higher than that of the other, 1-phenyl-1-metaxylyl-ethane. In KMC-A, the two main components of di-isopropylnaphthalene isomers a (the largest) and b seemed to be decomposed at a similar rate.

In Table 2, apparent Michaelis' constant (K_M), and maximum reaction velocity (V_m), assuming Michaelis-Menten's equation for the decomposition mechanism, are presented, though they are not exact values because the concentrations of the substrates examined were too few for exact calculation of the values.

DISCUSSION

Both SAS and KMC are commercial names. The chemical structures of the compounds are 1-phenyl-1-xylyl-ethanes and isopropyl-naphthalenes respectively. They were developed by Nippon Petrochemicals Co. Ltd. and Kureha Chemical Industry Co. Ltd., respectively, as new solvents in place of PCB. Their toxicities were, of course, studied by the investigators in the plants and in some universities that participated with the companies^{2,3)}. Since then the new solvents have been used in various industries. Non-carbon paper is their main use, and therefore the products are used widely not only in the plants but in general businesses.

The toxicity of the solvents was further investigated as one of the national studies in Japan¹⁾. Our first studies were performed in experimental animals, and then health examinations of workers in the plants were conducted with reference to the results obtained by the animal experiments. This paper is the first report of the animal experiments.

When 0.1 g of SAS-296 or KMC-A per kg of body weight was administered orally, large amounts of the substances appeared in the liver 2 hours after the dose, and disappeared within 24 hours. In the fat, there was a little accumulation after a single dose. Continuous dose for a month also caused a little accumulation of the substances in the fat, but the amounts in the fat did not affect the distribution and the amounts in the other organs.

The metabolism of the components of SAS-296 and KMC-A was investigated by using liver homogenate, and the apparent values of K_M and V_m were calculated. The results *in vitro* seemed to be adequate to explain the rapid disappearance in the organs. It may be concluded that neither SAS nor KMC accumulated in the organs other than in body fat and in subcutaneous fat.

In 1982, 10 years after our study, Addison *et al.*⁷⁾ showed the induction of hepatic mixed function oxidase activity in trout administered 0.03–0.1 g of 1-phenyl-1-xylyl-ethanes or di-isopropylnaphthalenes per kg of body weight. They also reported the accumulation of only 2 to 7% of the dose. This observation is consistent with our results, but the facts that the accumulation in rats was observed only in the fat and that the accumulated substances in the fat did not affect the distribution in the other organs should be considered in health control of workers.

Addison *et al.*⁷⁾ also indicated that all components of SAS accumulated in trout but some of the KMC components did not. In contrast our experiment showed that KMC-A seemed to accumulate more than SAS-296 in rats. The rate of decomposition of each component of KMC-A by liver homogenate was

nearly the same, but that of SAS-296 was not. The rate for 1-phenyl-1-orthoxylyl-ethane was larger than that for 1-phenyl-1-metaxylyl-ethane. The rate of decomposition of SAS-296 was higher than that of KMC-A. These facts are consistent with the observations that more KMC-A than SAS-296 is deposited in fat and that SAS-296 in fat is eliminated more easily than KMC-A. From Figs. 5 and 6, the half-life of KMC-A in fat can be seen to be about 7 days, and that of SAS-296 about 2 to 4 days. Yoshida and Kojima⁸⁾ showed in carp administered di-isopropylnaphthalenes a half-life of 56 hours at 25°C. Iwahara⁴⁾ reported a short half-life in mice administered KMC-A.

As metabolic products, a mixture of polar products has been assumed but not yet ascertained^{4,8)}.

The investigators in the plants showed already that decomposition of SAS and KMC in concentration of 30 ppm by activated sludge was accomplished in 5 to 10 days, though Sumino⁶⁾ reported di-isopropylnaphthalenes as contaminants of estuarine mud and biota.

On the basis of these facts, we can presume that the accumulation of SAS or KMC in the living body except in the fat will be negligible, and serious pollution of the general environment will not occur.

REFERENCES

- 1) Hasegawa, H., Sato, M. and Tsuruta, H. (1973). *Special Report on the Effects on Human Health and Chronic Toxicity of PCB and Other Pollutants, to Prevent the Pollution from Them*. p. 139, Science and Technology Agency of Japan, Tokyo.
- 2) Kureha Chemical Industry Co. Ltd. (1972). *Research data on KMC oil performed by Dr. Kawai et al. in Tokyo Dental University*.
- 3) Nippon Petrochemicals Co. Ltd. (1972). *Research data on SAS performed by professor Saito et al. in Nippon University*.
- 4) Iwahara, S. (1974). *Nat. Def. Med. J. (Japan)*, **21**, 273.
- 5) Kojima, S., Kagawa, M., Suzuki, R., Horio, M. and Tanaka, Y. (1978). *Chem. Phar. Bull.*, **26**, 3007.
- 6) Sumino, K. (1977). *Arch. Environ. Contam. Toxicol.*, **6**, 365.
- 7) Addison, R.F., Zinck, M.E., Willis, D.E. and Wrench, J.J. (1982). *Toxicol. Appl. Pharmacol.*, **63**, 166.
- 8) Yoshida, Y. and Kojima, H. (1978). *Chemosphere*, **7**, 491.