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Exposure to 2,2-Dichloro-1,1,1-trifluoroethane (HCFC-123) and Acute Liver Dysfunction: A Causal Inference

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Abstract: Exposure to 2,2-Dichloro-1,1,1-trifluoroethane (HCFC-123) and Acute Liver Dysfunction: A Causal Inference: Toru TAKEBAYASHI, *et al.* Department of Preventive Medicine and Public Health, School of Medicine, Keio University—Acute liver dysfunction has been reported among workers repeatedly exposed to 2,2-dichloro-1,1,1-trifluoroethane (HCFC-123), a substitute for trichlorofluoromethane. Causality between occupational exposure to HCFC-123 and liver dysfunction was examined. Levels of exposure to HCFC-123 were estimated retrospectively by reproducing working conditions and by a job record survey. Health surveillance, including liver function and subjective symptoms, was done when two workers first complained of ill health. The mean HCFC-123 concentration in air was more than 200 ppm with a peak concentration of about 1,000 ppm in a processing area where HCFC-123 was used. HCFC-123 of 18–24 ppm was detected in the adjunct areas where HCFC-123 vapor was not generated. Workers (n=14) were then classified into high (n=5) and low (n=9) exposure groups according to the estimated exposure level, which was confirmed by determination of urinary trifluoroacetic acid. Mean serum AST and ALT levels were 236 IU/l and 476 IU/l among the high-exposed workers, and exceeded 500 IU/l in three workers. Various types of symptoms involving the central nervous system and digestive organs, and irritation of the mucous membrane, were also experienced. The degree and prevalence of these health effects were higher in the high exposure group, which indicates the exposure-effect and exposure-response relationships. The consistency and temporality of the relationship between HCFC-123 exposure and the observed health effects were also confirmed. We conclude that repeated exposure to high concentrations of HCFC-123 for no more than five weeks causes acute severe liver dysfunction with various symptoms in humans. Biological plausibility must be clarified to confirm the causality.
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Concerns about the depletion of the stratospheric ozone layer have been increasingly widespread, and several replacements for chlorofluorocarbons (CFCs) are now in use. Extensive toxicological evaluations of these CFC replacements have been performed by the Programme for Alternative Fluorocarbon Toxicity Testing (PAFT)[1–3]. 2,2-Dichloro-1,1,1-trifluoroethane (HCFC-123; CAS No. 306-83-2) is a substitute for trichlorofluoromethane (CFC-11) used mainly as an industrial refrigerant. It is a colorless light-ether-odor liquid with a boiling point of 27.6°C at 1 torr, and its ozone depletion potential is 0.02. In 1996, 500 tons of HCFC-123 were shipped in Japan according to the statistics of the Japan Fluorocarbon Manufacturers Association. Results of the PAFT experiments indicated that the inhalation toxicity of HCFC-123 was low even in a 2-year bioassay in rats at concentrations of 300–5,000 ppm[2]. In 1997, however, Hoet *et al.* first reported an epidemic of liver disease among workers who had been accidentally and repeatedly exposed to mixtures of HCFC-123 and 2-chloro-1,1,1,2-tetrafluoroethane (HCFC-124)[4].

The second incident, occurring in Japan, which was

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reported by our research team[5], took place in a workplace that was set up in August 1997 to produce a small container (ca. 15 m*l* in volume) that included HCFC-123 as a refrigerant. The manufacturing process consisted of a preparation process, a refrigerant enclosure process in which HCFC-123 liquid was enclosed in a container, and an inspection process. HCFC-123 consumption increased in mid-September of 1997 when the refrigerant enclosure process started, and about a month later liver dysfunction was observed among workers who had been repeatedly exposed to HCFC-123. The aim of this study is to examine the causality between occupational exposure to HCFC-123 and the liver dysfunction we observed, with particular focus on the exposure-effect and exposure-response relationships.

Subjects and Methods

Estimation of exposure level in the workplace

The result of a hygienic survey revealed that there was no major chemical, physical or biological hazard other than HCFC-123 exposure in the workplace where the incident occurred. Because the work environment was immediately improved after the incident by the installation of effective local exhaust ventilation systems, it was difficult to retrospectively determine the degree of exposure to HCFC-123 at the time of the incident. The exposure level was therefore estimated by employing the following procedures: (1) estimation of HCFC-123 concentration in air by a field experimental survey reproducing the work conditions as of September-October; and (2) a job record survey on working hours within the refrigerant enclosure process area from 8 September to 12 October. The latter results were further confirmed by an interview with an industrial physician. In addition, the urinary concentration of trifluoroacetic acid (TFA), a major metabolite for HCFC-123[6], was determined for some of the workers whose urine samples had been taken on 14 October and stored at $-20°C$. Methods of measurement for HCFC-123 in air and TFA in urine are described in detail in the accompanying paper[7].

In the field survey, the working conditions could not be reproduced precisely because hygienic improvements had already been initiated. HCFC-123 was therefore vaporized under conditions similar to those in the real workplace environment. The volume of HCFC-123 vaporized was 2,798 g over the 6-hour experimental survey. This was the sum of the estimated HCFC-123 consumption in each constituent process in the refrigerant enclosure process on the day of maximum production of containers prior to the incident; i.e., 792 g for the injection process, 1,610 g for the leakage check process, and 396 g for the deaeration process. Because the leakage check was an intermittent process, HCFC-123 was vaporized in 200 min. The enclosure process area (ca. 83 m^3 in volume) was separated from both the preparation area and the inspection process area where HCFC-123 was not in use, but HCFC-123 spread diffusely into these adjacent areas through an opening in a partition. The HCFC-123 concentration in air was measured both in the enclosure process area and the neighboring areas throughout the experiment. A sampling point was set for each process where workers were presumably exposed to HCFC-123 during work.

Health surveillance and exposure-effect and exposure-response relationships

Fourteen workers (12 males and 2 females) were engaged in the processes, and blood and urine samples were collected from all of them on either 13 or 14 October, 1997, when two workers first complained of ill health. Blood biochemical indices examined were asparate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyltranspeptidase (γ-GTP), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), total and direct bilirubin, and the presence of hepatitis B antigen and hepatitis A and C antibodies. Hematological indices were also measured. The prevalence of subjective symptoms was surveyed through a self-administered questionnaire[8, 9] and an interview with the industrial physician. Periodical health check-ups continued until the workers' health states were confirmed to be normal.

The workers were classified into two groups, a high exposure group and a low exposure group, according to the results of the field experimental survey and the job record survey. The mean values of the blood indices or the prevalence of subjective symptoms were then compared between these two groups by Student's *t*-test with log transformation or Fisher's exact method when appropriate to examine whether the outcome variables changed in relation to exposure levels.

Results

Estimation of exposure level to HCFC-123

HCFC-123 concentrations in air were extremely high within the refrigerant enclosure process area (Table 1). During the 6-h experiment, geometric means for the injection process and the deaeration process were more than 200 ppm, with peak concentrations of 1,125 ppm and 817 ppm, respectively. The HCFC-123 concentration fluctuated and elevated when the leakage check process started. Workers were also exposed to 18–24 ppm of HCFC-123 even in the adjunct processes where HCFC-123 vapor was not generated. Temperature and relative humidity during the experiment were 18–22°C and 22–38%, respectively. The former range was similar to that in September or October, but the latter was somewhat lower.

The job record survey revealed that six out of 14 workers had been mainly engaged in the refrigerant

Table 1. HCFC-123 concentrations in air (ppm) over the field experiment reproducing the workplace condition as of September-October, 1997

Process	Arithmetic mean	Geometric mean	Range
Refrigerant enclosure process			
Injection process	405	225	45–1125
Deaeration process	376	227	29–817
Leakage check process	149	75	24–519
Adjunct process			
Preparation process	23	18	4–41
Injection process	28	24	5–40

n=9 for each process

Table 2. Results of the biochemical tests on 13–14 October, 1997 when the incident was identified

Group		AST [IU/*l*] (10–40)	ALT [IU/*l*] (5–45)	γ-GTP[IU/*l*] (M≦50/F≦30)	ALP [IU/*l*] (50–250)	LDH [IU/*l*] (208–442)	t-Bil [mg/d*l*] (0.2–1.1)	d-Bil [mg/d*l*] (≦0.4)
High (n=5)	geometric mean (GSD)	236 (3)*	476 (3)*	99 (2)*	309 (1)*	555 (1)*	1.8 (2.8)	0.7 (3.6)
	range	45–822	61–1715	26–290	237–462	359–808	0.5–7.5	0.2–4.4
	prevalence ¶	5/5*	5/5*	4/5	4/5	3/5*	2/5	2/5
Low (n=9)	geometric mean (GSD)	25 (1)	33 (2)	27 (2)	196 (1)	349 (1)	0.5 (1.3)	0.1 (1.4)
	range	21–30	18–70	15–98	143–295	300–405	0.4–0.9	0.1–0.2
	prevalence ¶	0/9	3/9	2/9	2/9	0/9	0/9	0/9

¶, a case was considered as prevalent when the test result exceeded the upper limit of the normal range which is indicated in the parenthesis under the item name. GSD, geometric standard deviation; *, p≦0.05 compared to the low group.

enclosure process with working hours of 50 h or more for the period of 8 September to 12 October. Two of them worked for more than 100 h in this period. The other six were mostly engaged in the preparation or inspection process; four worked for 20–50 h and two worked less than 20 h in the enclosure process. The other two were in charge of equipment maintenance.

Because exposure to HCFC-123 was much higher among workers involved in the refrigerant enclosure process than in those engaged in the adjunct processes, five workers who worked at the enclosure process for 50 h or more were assigned to the high exposure group, excluding one worker who actually worked largely outside of the enclosure process. The remaining 9 workers were taken as the low exposure group. This classification was further confirmed by an interview with the industrial physician.

Urinary TFA was measured in 10 workers, three from the high and seven from the low exposure group. Medians (range) of the TFA concentration for the high and low exposure group were 21.7 (2.0–29.3) and 4.0 (2.0–9.8) mg/g·creatinine, respectively. These urine samples were collected on 14 October, which was at least two days after the last exposure, but the existence of TFA in urine even in the low exposure group indicated that all workers had been exposed to HCFC-123 to a certain degree. The higher TFA concentration in the high exposure group also supported the field-survey finding that the HCFC-123 concentration was much higher within the refrigerant enclosure process area.

Health surveillance and exposure-effect and exposure-response relationships

Table 2 shows the results of the biochemical tests. AST and ALT levels of three workers in the high exposure group exceeded 500 IU/*l*, which indicated that severe liver damage had occurred among them. Clearly, the degree and prevalence of liver dysfunction were higher in the high exposure group. Various types of symptoms were experienced by the workers (Table 3), and could be classified into three groups: symptoms related to the central nervous system, those related to the digestive organs, and those related to irritation of the mucous membrane during HCFC-123 exposure. Prevalence of the subjective symptoms also increased in an exposure-dependent manner.

By the end of November, the health condition of all workers was confirmed to be normal. All tests were

Table 3. Number of workers who experienced symptoms with relation to HCFC-123 exposure

Symptoms	High (n=5)	Low (n=9)
Symptoms experienced since the refrigerant enclosure process started		
Digestive organs		
Abdominal pain	5 *	0
Stomach upset or loose bowels	5 *	0
Poor appetite	4 *	0
Nausea	3 *	0
Vomiting	2	0
Any digestive symptoms	5 *	0
Central nervous system (CNS)		
Heavy feeling in the head	5 *	2
Headache	3	2
Light-headedness	2	0
Muddle-headedness	0	2
Any CNS symptoms	5	4
Symptoms experienced during work in the refrigerant enclosure process		
Heavy feeling in the head	4	2
Dizziness	2	0
Headache	0	2
Discomforting smell	2	3
Irritation of eye or throat	2	1

*, $p \leqq 0.05$ compared to the low group.

negative for hepatitis viruses except for one worker who showed positive serology for the hepatitis A antibody. None of the workers had a history of drug- or alcohol-related liver diseases, and there were no changes in hematological indices in any of the workers.

Discussion

The results of this study indicate that repeated exposure to high concentrations of HCFC-123 for no more than five weeks causes acute severe liver dysfunction in humans with symptoms including various effects on the digestive organs and the central nerve systems. First, we note that these findings are consistent with the report of Hoet *et al.*[4] in which repeated exposure to a mixture of HCFC-123 and HCFC-124 resulted in severe liver damage. Though the exposure level was unknown in their report, the observed degree and clinical course of liver impairment were very similar to those seen here. Secondly, higher exposure to HCFC-123 in the refrigerant enclosure process resulted in more severe liver effects, by which exposure-effect and exposure-response relationships between the HCFC-123 exposure level and liver functions were indicated. The detection of a higher TFA concentration in urine among workers mainly engaged in the refrigerant enclosure process also supported these relationships. Moreover, exposure to HCFC-123 preceded the development of liver dysfunction, because the liver functions of some affected workers had been almost within normal range at the time of a periodical health checkup in April, 1997 (data not shown). Consistency, exposure-response relationship, and temporality are essential in elucidating causality in epidemiology. Effects of occupational exposure to HCFC-123 on the liver seen here were therefore likely to be causal.

The reason for the discrepancy between the toxicological evaluation of HCFC-123 and the observed liver dysfunction in humans remains unclarified. The LC_{50} for 4-h acute inhalation has been reported as 28,400 ppm in Chinese hamsters, and as 35,000 ppm in Sprague-Dawley rats[1]. The PAFT experiments evaluating the inhalation toxicity of HCFC-123 have been performed mainly in rats. Rusch *et al.* reported a series of four subchronic studies in which 300–20,000 ppm of HCFC-123 were exposed for 1–3 months[1]. While exposure to 300 ppm HCFC-123 for 90 days induced hepatic peroxisome proliferation and decreased cholesterol and triglyceride levels, and exposure to 1,000–5,000 ppm resulted in an increase in relative liver weight and a decrease in body weight, exposure to as high as 20,000 ppm for 4 wk did not induce marked signs of toxicity, with the exception of an increase in the serum clinical enzymes, AST and ALT, at 20,000 ppm. Findings of histopathological examinations were also minimal. They

thus concluded that HCFC-123 had a low order of subchronic toxicity. Even in the 2-yr inhalation study of HCFC-123[2], non-tumorgenic effects of HCFC-123 were few, and included decreased body weight at an HCFC-123 level of 300 ppm. In the evaluation of carcinogenic potential, increases in the incidence of some benign adenomas and in hepatic β-oxidation were observed at HCFC-123 of 300 ppm or more.

In contrast to the results of the PAFT experiments, a single exposure to 1,000 ppm HCFC-123 for 4 h in guinea pigs caused histopathological changes: hepatic degeneration and necrosis with increases in serum AST and ALT[10]. 1-Bromo-1-chloro-2,2,2-trifluoroethane (halothane), a well-known hepatotoxic chemical, has a structure very similar to that of HCFC-123. The oxidative pathway, catalyzed by CYP2E1, was suggested to be the major metabolic pathway by which both chemicals produce trifluoroacetylchloride, which then reacts with water to form TFA or bind covalently to hepatic protein, thereby inducing hepatic necrosis[11, 12]. The detection of trifluoroacetyl-protein adducts in liver biopsy samples, of an autoantibody that reacts with human liver P58 or CYP 2E1 in serum in the report of Hoet *et al.*[4], and of high concentrations of TFA in urine in our study indicate that oxidation by CYP 2E1 plays an important role in the metabolic activation of HCFC-123 in humans. A guinea pig model has been shown to be more sensitive and more appropriate than a rat model to evaluate halothane hepatotoxicity in humans[13, 14]. In the case of HCFC-123, the capacity of the human liver to bioactivate HCFC-123 to hepatotoxic metabolites via the oxidative pathway has been shown to be much higher than that in rats[11] *in vitro*. Toxicological evaluation of HCFC-123 should therefore account for the possibility of a species difference in the biotransformation pathway.

In conclusion, repeated exposure to HCFC-123 causes acute liver dysfunction involving various subjective symptoms. The working durations examined here were no more than five weeks, and the estimated mean concentrations of HCFC-123 in the refrigerant enclosure process exceeded 200 ppm, with peak concentrations above 1,000 ppm. Consistency, temporality, and exposure-response relationship were established, but the biological plausibility must be further examined to confirm the causality of HCFC-123 exposure on liver dysfunction in humans.

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