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Abstract

Objectives—To investigate the possible effects of occupational exposure to the nematocide *cis*-1,3-dichloropropene (*cis*-DCP) on function of the kidney and liver in the starch potato growing region in The Netherlands.

Methods—The study involved 13 commercial application workers exposed to *cis*-DCP for 117 days, and 22 matched control workers. The inhalatory exposure of the application workers was estimated from biological monitoring data. All workers collected urine and serum samples before, during, and after the fumigation season for monitoring of variables for kidney and liver function. Renal effect variables were alanine aminopeptidase (AAP), N-acetyl- β -D-glucosaminidase (NAG), retinol binding protein (RBP), and albumin (ALB) in urine, and β_2 -microglobulin (β_2 M-S) and creatinine in serum (Creat-S). Liver variables were alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), γ -glutamyltranspeptidase (GGT), alkaline phosphatase (ALP), and total bilirubin (TBIL) in serum and the urinary ratio of 6- β -hydroxycortisol to free cortisol (β OHC/COR).

Results—The geometric mean exposure of the application workers was 2.7 mg/m³ (8 hour time weighted average (8 hour TWA)); range 0.1–9.5 mg/m³. No differences were found between the values of the renal effect variables or the liver variables of the exposed group and the control group, except a lower urinary ratio of β OHC/COR in the exposed group. This was not considered to be related to the exposure to *cis*-DCP. No dose-effect relations were found between the exposure indices and the effect variables.

Conclusions—The present study does not provide evidence that occupational exposure to *cis*-DCP in the starch potato growing region causes adverse effects on the kidney or liver at 8 hour TWA exposure concentrations below 9.5 mg/m³ (2 ppm).

(Occup Environ Med 2000;57:745–751)

Keywords: *cis*-1,3-dichloropropene; occupational exposure; kidney; liver

In The Netherlands, 1,3-dichloropropene (DCP) is used mainly as a soil fumigant for the eradication of soil nematodes in the starch potato growing region and the flower bulb culture. Formerly the commercially available

DCP fumigant included both *cis* and *trans* isomers^{1,2}; the DCP fumigants presently available, Telone-*cis* and Nematrap, consist of more than 95% of the more active isomer *cis* DCP.

In rats, the major route of DCP metabolism is conjugation with glutathione, catalysed by hepatic glutathione transferase.³ The glutathione conjugate is further metabolised into the mercapturic acid metabolite N-acetyl-S-(*cis*-3-chloro-2-propenyl)-L-cysteine (*cis*-DCP-MA). This mercapturic acid metabolite is excreted in urine and is suitable for biological monitoring purposes.^{1,4} This was confirmed by Brouwer *et al.*⁵

Torkelson and Oyen found nephrotoxic and hepatotoxic effects in rats and guinea pigs exposed to 227 mg/m³ *cis*-DCP or *trans*-DCP, in 19 7-hour exposures for a period of 28 days.⁶ Slight to marked effects on liver and kidney were found in rats and guinea pigs exposed to 50 mg/m³ DCP, in 27 7-hour exposures for 39 days. Exposure to 14 mg/m³ for 6 months induced “very slight cloudy swelling of the renal tubular epithelium”. However, the effects in their study were not confirmed by other assays.^{7–10}

In a recent occupational health study, slight nephrotoxic and hepatotoxic effects were found in application workers exposed to *cis*-DCP or *trans*-DCP. In 15 workers exposed to 0.3–9.4 mg/m³ DCP, increased excretions of the renal effect variables N-acetyl- β -D-glucosaminidase (NAG)¹ and retinol binding protein (RBP)¹¹ were found. Albumin (ALB) in urine was not increased. Stott *et al.*¹² argued that the NAG excretion found by Osterloh *et al.*¹ may have been a result of the stimulation of exocytosis or an increase of the NAG activity in the kidney, rather than an indication of nephrotoxicity.

In an exploratory study in the flower bulb culture, Brouwer *et al.*⁵ investigated renal and liver effect variables before and after the application season in 14 workers exposed to 8 hour time weighted average (8 hour TWA) concentrations of *cis*-DCP or *trans*-DCP of 1.9–18.9 mg/m³ for 4–37 days. The occupational exposure limit (OEL) for DCP in The Netherlands (5 mg/m³) was exceeded in 30% of the observed working days. Renal effect variables studied were alanine aminopeptidase (AAP), β -galactosidase (β GAL), RBP, β_2 -microglobulin (β_2 M), and ALB in urine and creatinine in serum (Creat-S) and β_2 M-S in serum; liver variables were alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), γ -glutamyltranspeptidase (GGT), alkaline phosphatase (ALP), lactate dehydroge-

Table 1 Descriptive statistics of the study groups

Variable	Controls		Exposed	
n	22		13	
Age (y, median (range))	34 (22–53)		31 (24–51)	
Body mass (kg, median (range))	89 (62–108)		83 (75–100)	
Height (m, median (range))	1.80 (1.65–1.95)		1.85 (1.70–1.94)	
Employment (y, median (range))	14 (1–33)		11 (2–31)	
Smoking behaviour (cigarettes/day, n (%)):				
0	8	36	6	46
<10	3	14	0	0
10–20	9	41	7	54
>20	2	9	0	0
Alcohol consumption (g/day, n (%)):				
0	3	14	2	15
<20	16	72	10	77
20–50	3	14	1	8

nase (LD), and total bilirubin (TBIL) in serum. After the application season a slight effect on renal effect variables was found: RBP and ALB were increased, but Creat-S was slightly decreased. The liver variable TBIL was decreased at the end of the season. In combination with an increase in GGT this indicated moderate hepatic enzyme induction. However, the cause-effect relation of these results has been contested because of the absence of a control group.¹³

The purpose of the present study was to investigate whether exposure of commercial application workers to *cis*-DCP in the starch potato growing region causes effects on liver and kidney in concordance with the results of the exploratory study of Brouwer *et al* with the racemic mixture in the flower bulb culture.²

The present study was designed to include an appropriate control group and monitoring of liver and kidney variables before and during the application season, and up to 1 month after the end of application. Also, a possible dose-effect relation between exposure to *cis*-DCP and the effect variables was investigated.

Study group and methods

STUDY GROUP

The study design allowed for a maximum of 22 application workers and 22 controls. Exclusion criteria were disorders of bile, the liver or kidney, diabetes, or hypertension during the past 5 years, and current intake of drugs with known nephrotoxic or hepatotoxic side effects.

All known fumigation firms in the northern part of The Netherlands were informed about the study by letter and invited to an information meeting. Thirty one of the estimated total of 35 application workers were willing to participate. All of them gave their informed consent before inclusion in the study. Application workers were excluded for diabetes (n=1) and treated hypertension (n=2). Application workers who expected to fumigate for less than 5 days during the season (n=5) were not included in the study. Of the remaining 23

application workers one person was excluded from analysis of liver and renal effect variables because of the presence of glucose in the urine. This application worker was included in the exposure assessment because he was one of the few subjects to use a compressed air system to prevent spillage.⁵

Lorry drivers (n=47), who transport potatoes from the farms to three potato processing factories in the northern part of The Netherlands, were invited to participate in the study as controls. People with disorders of bile (n=2) or the kidney (n=1), diabetes (n=1), treated hypertension (n=3) and intake of drugs with known nephrotoxic or hepatotoxic side effects (n=1) were excluded. For logistical reasons lorry drivers who transported potatoes to one of the three factories (n=14) were excluded. Of the remaining 25 lorry drivers three aged 40–49 were excluded to improve matching for age. The final control group comprised 22 people.

Due to bad weather conditions and economic reasons only 13 of the selected 22 application workers fumigated with DCP during the autumn of 1993. Thus 13 application workers were monitored for liver and renal effects. Descriptive statistics of the study groups are shown in table 1.

COLLECTION OF URINE AND BLOOD SAMPLES

Urine and serum samples were used to measure effects on the kidney and liver. Each participant collected eight overnight urine samples on Tuesdays, Wednesdays, or Thursdays in the period from August to December (fig 1). The fumigation season started in week 34 and continued until week 45. Samples were collected before (weeks 32 and 33), during (weeks 38, 41, 43, and 45), and after (weeks 47 and 50) the fumigation season.

Urine was collected in 500 ml polyethylene containers to which 0.1 ml NaN₃ (0.08 mol/l) was added. The collection time and period of each urine sample were entered on a separate form. Venous blood samples were collected five times in the collection period (fig 1). Blood was allowed to clot and serum was separated by centrifugation.

A complete set of urine (n=104) and serum (n=65) samples was obtained from all 13 fumigators. In the control group, urine samples were missing in week 41 (n=2) and weeks 43, 45, and 50 (each n=1); 171 urine samples were obtained. Serum samples in the control group were missing in week 41 (n=2) and weeks 45 and 50 (each n=1); 106 serum samples were obtained.

MEASUREMENT OF RENAL EFFECT VARIABLES

Portions of the urine samples were eluted on Sephadex G-25M columns (Pharmacia LKB

Week	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50
Urine collection	X	X					X			X	X	X		X		X			X
Serum collection	X									X				X		X			X
Fumigation			X		X	X	X	X			X	X	X	X					

Figure 1 Study design.

Biotechnology, Sweden) for the measurement of NAG and AAP. The NAG was measured by a modified method of Maruhn.¹⁴ The AAP was measured within 1 week after collection by the method of Jung and Scholtz.¹⁵ Separate portions of urine as well as the serum samples were frozen at -20°C for the other assays. RBP, ALB, and $\beta_2\text{M-S}$ were measured by latex immunoassay.¹⁶ Creat-S was measured by a modified method of Jaffé (Merck, Germany). All analyses were performed on an EPOS 5060 automated analyzer (Merck Eppendorf, Germany). All samples were measured in duplicate and in random order. Samples with a concentration or activity below the lower detection limit were given a value of half the detection limit for statistical evaluation.¹⁷ Values of RBP in four samples were below the detection limit of 10 $\mu\text{g/l}$. Albumin values in 17 samples were below the detection limit of 0.66 mg/l . None of the other renal effect variables had values below the detection limits.

The duplicate precision within a run of the measurements of the renal effect variables was for AAP 3.4%, NAG 1.5%, RBP 6.4%, ALB 5.5%, $\beta_2\text{M-S}$ 6.0%, and Creat-U 1.5%. The precision between runs of the determinations was calculated for two internal quality control samples analyzed in each run. The activities or concentrations of the quality control samples and the precision between runs (in parentheses) were for AAP 5.20 (6.8%) and 15.7 (5.0%) U/l; NAG 1.88 (3.2%) and 5.66 (2.0%) U/l; RBP 77 (14.2%) and 189 (6.0%) $\mu\text{g/l}$; ALB 3.95 (4.8%) and 40.6 (6.8%) mg/l ; $\beta_2\text{M-S}$ 1.17 (2.2%) and 2.53 (1.7%) mg/l ; Creat-U 0.89 (2.8%) and 2.43 (3.8%) g/l .

The activities or concentrations of the urinary renal effect variables were divided by the concentration of creatinine in urine (Creat-U) to adjust for dilution of the urine samples.

There are no data available suggesting an interference of *cis*-DCP or its metabolites with the analysis of these renal effect variables.¹⁸⁻²⁰

MEASUREMENT OF LIVER VARIABLES IN URINE OR SERUM

The urinary ratio of 6- β -OH-cortisol (βOHC) to free cortisol (COR) was measured for the assessment of cytochrome *P*-450III_A enzyme induction. The βOHC was measured by enzyme immunoassay (Stabiligen, Nancy, France). The COR was measured by a high performance liquid chromatography (HPLC) at the Laboratory for Endocrinology of the Academic Medical Center of the University of Amsterdam. The liver variables ALAT, ASAT, GGT, ALP, and TBIL were measured on a Hitachi 747 automated analyzer (Boehringer, Mannheim, Germany) with reagents from the same manufacturer at the Laboratory for Clinical Chemistry of the Academic Medical Center of the University of Amsterdam. In seven serum samples the values for ASAT (week 41 $n=2$; week 47 $n=1$; and week 50 $n=4$) were rejected because of haemolysis. The precision between runs of each of the liver variables in serum was less than 1.6%.

The βOHC values (in week 38 and 45) could not be measured in two samples due to lack of

sample material. The βOHC values in 89 urine samples were below the detection limit of 100 $\mu\text{g/l}$. The duplicate precision of the measurements of the liver variable βOHC was 13.0%. The precision between runs of the measurements of βOHC was calculated for one internal quality control sample, analyzed in each run. The concentration of the quality control sample and the precision between runs were: 161 and 18.0%, respectively. The concentrations of the quality control samples and the precision between runs (in parentheses) for COR were: 58.9 nmol/l (11.2%) and 123.6 nmol/l (8.3%).

There are no data available suggesting an interference of *cis*-DCP or its metabolites with the analysis of these liver variables.¹⁸⁻²⁰

DOSE INDICES

To study the relation between dose and effect, the design should take into account an effect period: this is the period after which an effect becomes noticeable after the start of exposure and remains detectable after discontinuation of exposure. Biological effect monitoring should be performed during this period to minimise bias. The half life of elimination of *cis*-DCP-MA, corrected for creatinine, is 5.3 hours.⁴ Renal effects of the racemic DCP mixture were found within 24 hours after exposure.¹¹ In our exploratory study, both renal and liver effects were found after the application season, which suggests an effect period up to several months.² For other nephrotoxic substances—such as *cis*-platin—renal effect variables were found to be increased for a period of 5 days,^{21 22} and up to 2 weeks.²³ No other published examples of effect periods for liver variables were found.

Based on this limited information, the assumption was made that possible renal or hepatic effects, due to exposure to *cis*-DCP, will occur within a period of 2 weeks, with the maximum effect occurring within the first week after exposure. Thus, for each urine or serum sample of all participants the number of days with exposure to DCP preceding the sample collection were used as dose indices. These indices were measured as the sum of the number of fumigation days during 1 (DI1) or 2 (DI2) weeks preceding collection of the urine or serum samples. For 275 urine samples dose indices DI1-U and DI2-U could be calculated. The number of serum samples with dose indices DI1-S and DI2-S was 171.

CALCULATIONS AND STATISTICS

Non-normal distributions of variables (AAP, NAG, RBP, ALB, ALAT, GGT, and βOHC /COR) were transformed logarithmically. Two sided *t* tests were used to compare the means of the renal effect and liver variables on all collection days. The presence of dose-effect relations was studied with repeated measurement analysis. Repeated measurement analysis was performed with the general linear model procedure of SAS 6.11 for Windows.²⁴ For urine results an average baseline was calculated from results of weeks 32 and 33. The renal and liver variables in urine, collected during or immediately after exposure (weeks 38, 41, 43, and 45) were compared with the baseline

results. For serum variables weeks 41 and 45 were compared with baseline results of week 32.

Results

EXPOSURE TO *cis*-DCP

The exposure of the commercial application workers to *cis*-DCP is described extensively elsewhere.³ In summary, the 13 application workers performed fumigation on a total of 117 days. In weeks 40 and 41 fumigation was not possible due to heavy rainfall. During the last 2 weeks of the season, fumigation occurred on only 56% (n=66) of the days. The mean (SD) daily exposure time was 521 (230) minutes.

The geometric mean (range) 8 hour TWA average exposure—estimated from the biological monitoring data—was 2.7 (0.1 - 9.5) mg/m³. The Dutch OEL (5 mg/m³) was exceeded on 25 days (21%).

RENAL EFFECT VARIABLES

The means (SDs) of the activities or concentrations of the renal variables AAP, NAG, RBP, ALB, β_2 M-S, and Creat-S are shown in fig 2. No differences were found between the values of any of the renal effect variables of the exposed group and those of the control group. The absence of differences persisted through-

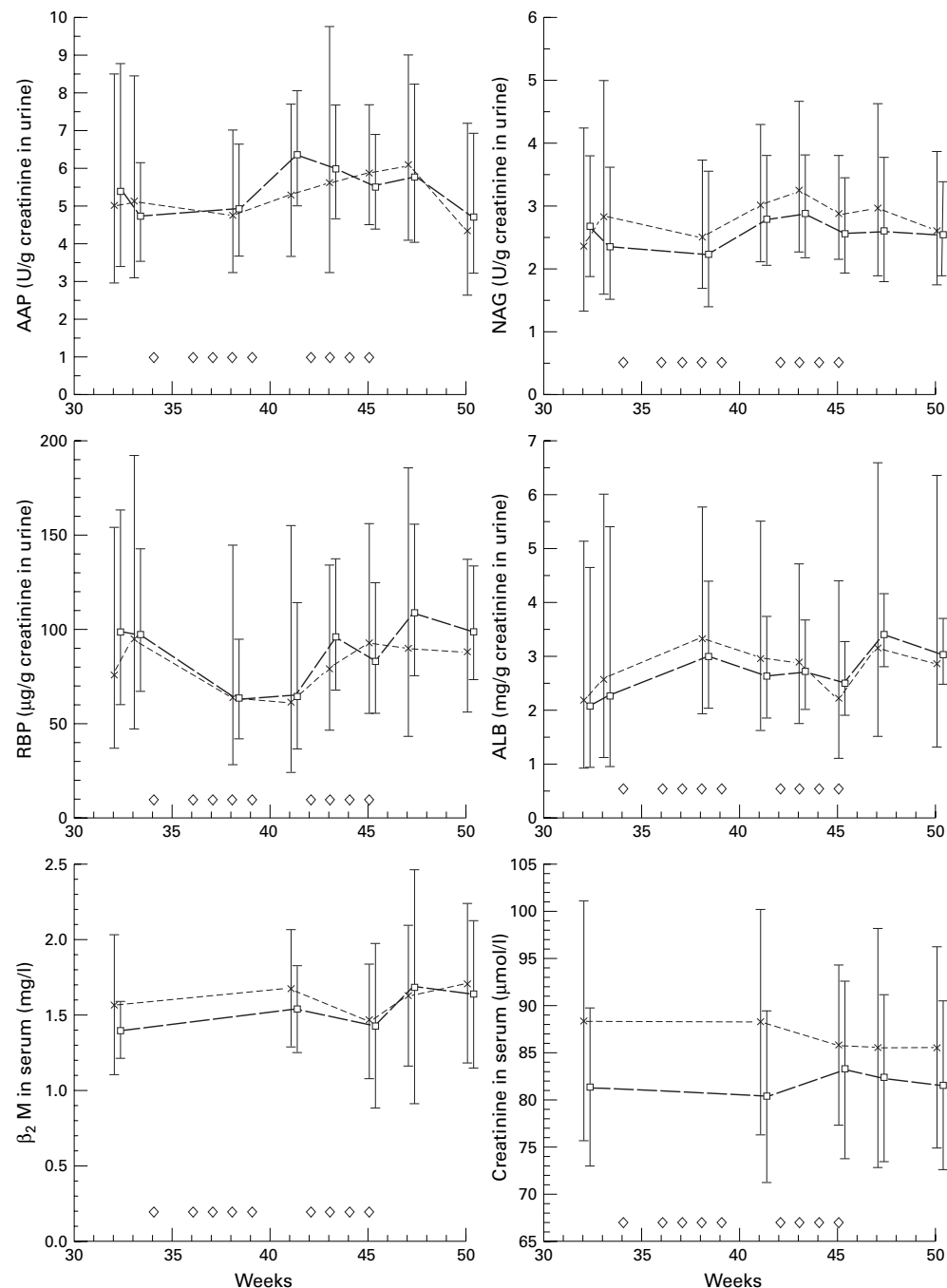


Figure 2 Means (SDs) of the renal effect variables AAP \ddagger , NAG \ddagger , RBP \ddagger , ALB \ddagger , β_2 M-S \ddagger , and Creat-S \ddagger in the exposed (\square) and control (\times) workers, before, during and after the fumigation season. \diamond Indicates weeks with fumigation activities; \ddagger geometric mean (SD); \ddagger arithmetic mean (SD).

out the whole study period, before, during, and after the fumigation season. Repeated measurement analyses confirmed the absence of effects related to exposure. The renal effect variables did not exceed reference values.

LIVER VARIABLES

The means (SDs) of the concentrations of the liver variables in serum ALAT, ASAT, ALP, GGT, TBIL, and the urinary variable β OHC/COR are shown in figure 3. No differences were found between the values of the serum variables of the exposed group and those of the control group. The liver function variable β OHC/COR

in urine was lower in the exposed group than in the control group in weeks 32, 41, 47, and 50. This difference seemed to persist throughout the whole study period. Repeated measurement analysis did not show exposure related changes in the liver variables. The values of the liver variables were not outside reference values.

DOSE-EFFECT RELATIONS

The dose indices comprise the number of fumigation days before effect monitoring. The frequency distributions of the dose indices DI1 and DI2 for the urinary and serum samples of the exposed and control group are shown in

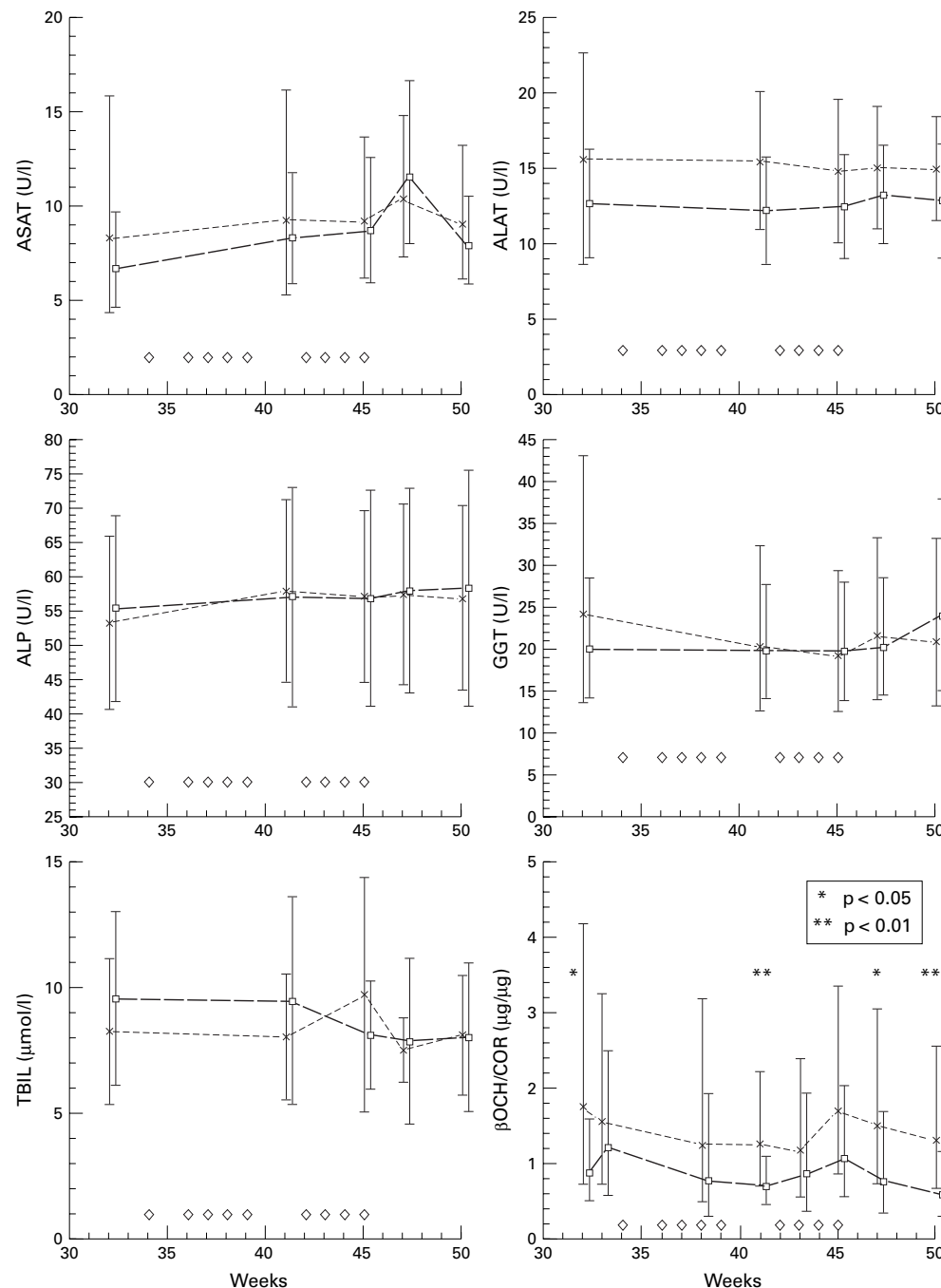


Figure 3 Means (SDs) of the liver parameters ALAT \ddagger , ASAT \ddagger , ALP \ddagger , GGT \ddagger , TBIL \ddagger , and β OHC/COR \ddagger in the exposed (□) and the control (x) workers, before, during, and after the fumigation season. ◇ Indicates weeks with fumigation activities; *p < 0.05; **p < 0.01; \ddagger geometric mean (SD); \ddagger arithmetic mean (SD).

Table 2 Frequency distributions of the dose indices DI1-U, DI2-U, DI1-S, and DI2-S

	Fumigation days (n)										
	0	1	2	3	4	5	6	7	8	9	10
DI1-U	257	6	4	1	5	1	1	0			
DI2-U	240	12	6	5	4	5	0	1	0	1	1
DI1-S	162	2	0	1	4	1	1	0			
DI2-S	146	8	3	4	5	3	0	0	0	1	1

The dose indices are comprised of the number of fumigation days during 1 (DI1) or 2 (DI2) weeks preceding collection of the urine or serum samples.

table 2. Regression analyses showed that none of the renal or liver variables were associated with the dose indices.

Discussion

No adverse effects of exposure to *cis*-DCP on the kidney and liver were found in this study. The only difference was a lower β OHC/COR ratio in the exposed group than in the control group. The β OHC/COR ratio is used to monitor hepatic cytochrome *P*-450III_A isoenzyme activity. This enzyme catalyses the oxidation of cortisol and several drugs, including rifampicin, nifedipine, and cyclosporin.²⁵ Increased enzyme activity can be shown by an increase in the β OHC/COR ratio. Our results do not suggest that *cis*-DCP induces cytochrome *P*-450III_A activity. On the contrary, the enzyme activity in the exposed group seems to be lower than in the control group. A decrease in β OHC/COR ratio would suggest either that *cis*-DCP is specifically metabolised by this form of *P*-450III_A and thus competes with cortisol, or that *cis*-DCP is an inhibitor of *P*-450III_A. In analogy, cimetidine, a known inhibitor of cytochrome *P*-450 isoenzymes, decreases β OHC excretion in humans.²⁶ The difference in the β OHC/COR ratio between the exposed and control group, however, persists throughout the whole study period and thus does not seem to be related to the exposure to *cis*-DCP. It has been shown that the β OHC/COR ratio in healthy people shows wide variability.²⁷ The difference in the β OHC/COR ratio can most likely be attributed to biological variability between the exposed and control group.

The other effect variables of both the exposed and control groups do not show significant trends during the study period. The variability in effect parameters over the whole period is similar in the control and the exposed group and thus favours a common, perhaps seasonal, cause. The variability in the mean values of the effect parameters in the exposed group do not seem to be related to the weeks in which fumigation was performed, and this is confirmed by the absence of dose-effect relations between the dose indices and the effect variables.

The dose indices comprised the number of fumigation days before effect monitoring. These indices do not take into account factors such as the height and duration of exposure during fumigation, and accumulation of *cis*-DCP or its metabolites. Therefore, the dose indices may be biased and may have weakened a possible association between the dose and effect variables. But in view of the absence of

differences between the control and the exposed group, it is not likely that a dose-effect relation between the exposure to *cis*-DCP and the renal and liver variables existed at the current levels of exposure.

We cannot exclude the possibility that a much larger study population could have showed small differences related to exposure in renal or liver variables. However, the biological relevance of such small differences can be questioned. Despite complex statistical analyses the current data do not show any indications of differences related to exposure. Moreover, all results were within the reference ranges.

The results of the present study contrast with the results of previous studies of Osterloh *et al.*,¹ Osterloh and Feldman,¹¹ and Brouwer *et al.*² Details of these studies and the main differences are summarised in table 3.

Since 1992 the mixture of *cis*-DCP and *trans*-DCP isomers has been replaced by a nematocide consisting of more than 95% *cis*-DCP. *Cis*-DCP has lower lethal dose for 50% of the animals (LD₅₀) than the *trans*-isomer, and is the more toxic isomer in animal experiments with short term high level exposures.²⁸ The absence of effects in the present study might suggest that the effects in the former studies were attributable to the *trans*-isomer. However, there are no animal or human data on low level long term exposure available to support this assumption.²⁸ Although data from short term high level exposure experiments cannot always be extrapolated to long term low level occupational exposures, it is not considered to be likely that the absence of effects in the present study is due to the change in formula of the presently available DCP nematocides.

The 8 hour TWA exposure to DCP in the present study is in the same range as in the studies of Osterloh *et al.*¹ and Osterloh and Feldman.¹¹ The exposure, especially the peak exposures, in the study of Brouwer *et al.*² in the flower bulb industry was higher. It cannot be excluded that these peak exposures were responsible for the effects found in application workers in the flower bulb industry.

The increases in NAG and RBP excretion in the studies of Osterloh *et al.*¹ and Osterloh and Feldman¹¹ were found on the same day as the exposure. In the current study most fumigation (45 days) was performed during week 45, and eight of the workers were exposed on the day before effect monitoring, but short term effects could not be found in any of the effect variables in this study.

The present investigation does not provide evidence that occupational exposure to *cis*-DCP during fumigation causes adverse effects on the kidney or liver at 8 hour TWA exposure concentrations below 9.5 mg/m³.

This investigation could not have been achieved without the complete cooperation of the commercial application workers and the lorry drivers, to whom we are very grateful. We express our gratitude to H du Jour, A van Schijndel, M van Gelder, and S Kuijper from the Coronel Laboratory for their technical assistance in the fieldwork and the laboratory analyses. We are grateful to E Endert and JPMC Gorgels for their help in the analysis of renal and liver function variables, EA van der Meulen, Erasmus University, Department of Epidemiology and Biostatistics, Rotterdam, and KM Bodner, DOW Chemical Company, Health

Table 3 Summary of the results of studies on effects of occupational exposure to DCP on renal or liver variables

Study groups	Exposure	Sampling for BEM	Effects	Reference
15 Male applicators	PM: median 1.70 mg/m ³ range 0.3–9.4 mg/m ³ cis-DCP and trans-DCP	Morning before exposure, noon, end of afternoon, late evening, and next morning urine; total 73 samples	Higher excretion rate of NAG in applicators with cumulative DCP in air >700 mg·min/m ³ or cis-DCP-MA >1.5 mg/day	1
16 Male applicators	PM: median 1.70 mg/m ³ range 0.3–9.4 mg/m ³ cis-DCP and trans-DCP	Morning before exposure, noon, end of afternoon, late evening, and next morning urine; total 73 samples	Higher excretion rate of RBP in applicators with cis-DCP-MA >1.5 mg/day; ALB not changed	11
14 Male applicators	PM: median 3.7 mg/m ³ range 1.9–18.9 mg/m ³ peak values up to 195 mg/m ³ cis-DCP and trans-DCP	Before and after the fumigation season, urine and serum samples	After season RBP and ALB increased, Creat-S decreased; no changes in β ₂ M-S, β ₂ M-U, AAP, β-galactosidase decrease of TBIL in combination with increase in GGT; no changes in ALP, ASAT, ALAT, lactate dehydrogenase	2
13 Male applicators; 22 matched controls	Based on BM data: geometric mean 2.7 mg/m ³ range 0.1–9.5 mg/m ³ peak values up to 15 mg/m ³ cis-DCP	Before, during and after the fumigation season, 8×urine and 5×serum samples	No differences in AAP, NAG, RBP, ALB, β ₂ M-S, and Creat-S between exposed and controls; no dose-effect relations. Lower βOHC/COH in the exposed group; No differences in ALAT, ASAT, ALP, GGT, and TBIL between exposed and controls; no dose-effect relation	Current study

PM = personal monitoring; BM = biological monitoring; BEM = biological effect monitoring.

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