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Concentrations of individual serum or plasma bile acids in workers exposed to chlorinated aliphatic hydrocarbons

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Abstract

Individual serum or plasma bile acid concentrations were measured by high performance liquid chromatography in two groups of workers with differing exposures: to hexachlorobutadiene (HCBD) and a mixture of other chlorinated solvents (SOLVENT) in study A; and trichloroethylene (TCE) in study B. Exposures to HCBD and TCE were associated with highly significant increases in a number of individual and summed bile acid measures, with a dose effect relation shown for HCBD. Exposure to SOLVENT was associated with significant decreases in three bile acid measures but this may have been due to misclassification of exposure. No association was found between any of the exposures and any of the standard tests of liver function. This preliminary study suggests that some chlorinated hydrocarbons are associated with raised bile acid concentrations in the blood of exposed workers. It may be that the changes in such concentrations reflect early and small disturbances of liver function. The significance and mechanism of the changes are yet to be determined.

Most occupational causes of liver dysfunction involve exposure to chemicals.[1] Many chemicals have unknown or uncertain hepatotoxicity, however, especially at the low exposures present in most modern workplaces.[2]

Currently most screening of liver function in the workplace involves the application of standard tests of liver function used in clinical medicine. These tests have, however, been criticised for lacking sensitivity and specificity in the workplace setting.[3] Serum bile acid concentrations have been proposed as more sensitive and specific indicators of liver dysfunction in workers.[4] Recent research in this area has concentrated on the possible hepatic effects of exposure to organic solvents in the workplace. Many studies have found raised serum bile acid concentrations in exposed workers despite normal values for standard liver function tests.[5–7] Exposure details and the choice of control groups in some of these studies were not optimal, however. Furthermore, the assays used to determine serum bile acid concentrations were not always able to discriminate between the individual bile acids. It is possible that one or more of the bile acids may be particularly sensitive to exposure to solvents and this may not be evident with these assays. Recent work with experimental animals supports this possibility.[8]

The aim of our study was to investigate individual bile acid concentrations in serum or plasma from workers exposed to chlorinated solvents.

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Materials and methods

Two studies were performed. Study A involved workers at a solvent production plant with potential exposure to a range of chlorinated solvents (mainly carbon tetrachloride (CTC) and perchlorethylene (PCE)), and to hexachlorobutadiene (HCBD), a chlorinated aliphatic hydrocarbon that has solvent properties but in this case was a byproduct of solvent production. Study B was conducted at a site manufacturing small appliances where trichloroethylene (TCE) was used for degreasing.

STUDY A

The study population consisted of all 53 members (all men) of the plant's workforce. Six subjects were excluded at a later stage because the blood samples were inadequate for analysis. Another subject who was taking antibiotics at the time was excluded. A further 11 subjects were excluded from the presented analysis because they had not fasted before the blood samples were taken, although qualitatively similar results were found when these subjects were included. This left 35 subjects for this analysis.

The plant was inspected with the site occupational hygienist. From the results of repeated environmen-

tal monitoring conducted by the plant it was possible to assign each worker to one of four degrees of exposure to HCBD. These ranged between 0 and 0·02 parts per million (ppm) (threshold limit value (TLV) = 0·02 ppm[9]). Routine monitoring had repeatedly shown that exposure to solvents was low (less than 1 ppm) but did vary with the task, allowing workers to be assigned to either a "lower" or "higher" solvent (SOLVENT) exposure group (TLV = 5 ppm for CTC; TLV = 50 ppm for PCE[9]).

Only limited information on characteristics of the subjects was available. This comprised age, duration of employment at the plant, and current use of medication.

STUDY B

The study population consisted of 22 volunteer subjects (21 men). All were fasted. Four were taking some form of medication on a regular basis and have been included in the analysis.

Subjects were easily divided into groups exposed and unexposed to TCE on the basis of their job tasks. Personal monitoring of workers in representative tasks performed six months before this study showed likely regular exposures of less than 5 ppm for most workers, with peak exposures for two workers possibly over 250 ppm on occasions. These two workers usually used some form of respiratory protection at times of high exposure (TLV = 50 ppm[9]).

Information was collected for each subject concerning age, duration of employment at the plant, use of medication, alcohol intake, and previous hepatic disease using a purpose designed self administered questionnaire.

COLLECTION PROCEDURES

Blood was collected at the beginning of a shift after an overnight fast. Tubes without anticoagulant were used for study A and heparinised tubes for study B. The serum or plasma was divided into several aliquots and frozen at −20°C until analysis.

ASSAY PROCEDURES

Serum (and plasma) bile acids were analysed by high performance liquid chromatography (HPLC) using a modification of the method previously described[10] that allows accurate determination of individual bile acids in serum. A Waters HPLC system with a Maxima-820 chromatography work station, Model 715 Ultra WISP sample processor and 470 scanning fluorescence detector (Waters Chromatography Division, Milford, MA, USA) were used, as well as a Blue Chip CM-6260 personal computer. Serum or plasma samples were spiked with the internal standard 23-nor-5β-cholanic acid-3a, 12a-diol (NCD; also called 3a-12a-dihydroxynor-cholanate or nordeoxycholic acid) and then mixed with cold acetone for deproteinisation before passing through a Sep-Pak C18 cartridge. The eluate was dried down at 60°C with a Jouan vacuum centrifugal evaporator, RC1010 (France). Standards and samples were both made up to 500 μl with acetonitrile and 20 μl was injected into the HPLC. For taurine conjugates samples were dried after the enzyme step and then taken straight to the derivative step.

Concentrations of cholic acid (CA), chenodeoxycholic acid (CDC), and deoxycholic acid (DC) were determined, along with their glycine (GC, GCDC, GDC) and taurine (TC, TCDC, TDC) conjugates.

Glycolithocholate (GLC), ursodeoxycholate (UDC), and tauroursodeoxycholate (TUDC) concentrations were also determined. From these values various summed measurements could be calculated—namely, concentrations of total glycine conjugates (GTOT); total taurine conjugates (TTOT); total cholate (CATOT); total chenodeoxycholate (CDCTOT); total deoxycholate (DCTOT), and total bile acids (BILTOT). Thus BILTOT is the addition of the individual bile acid concentrations measured by the HPLC analyser.

The standard tests of liver function used in this study were serum or plasma protein (Prot), albumin (Alb), and bilirubin (Bili) concentrations and alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and γ-glutamyl transpeptidase (GGT) activities. Serum or plasma cholesterol concentration (Chol) was also determined. Samples were analysed using an American monitor parallel analyser.

STATISTICAL ANALYSIS

The relations between outcome measures (bile acids or liver function tests) and exposure (HCBD, SOLVENT, or TCE) were examined, taking into account various confounding factors, using multiple linear regression techniques as programmed in the SAS system, Version 6·03 (SAS Institute, Cary, North Carolina, USA). The outcome variables were log transformed to normalise their distribution (with zero values set to 0·005 μmol/l). Residuals were checked to ensure that the underlying assumptions of the regression techniques were being upheld.

Results

STUDY A

Table 1 shows the mean age and duration of employment of subjects. Excluded subjects and included subjects had a similar mean duration of employment but excluded subjects were slightly younger. Neither age nor duration of employment differed greatly between exposure groups. Six workers had slightly raised GGT but they were equally apportioned between the four HCBD exposure groups and between the two SOLVENT exposure groups.

Table 1 Age and duration of employment of workers in study A

	Included (n=35) Mean (SD)	*Excluded (n=18) Mean (SD)*
Age (y)	39·1 (13·8)	32·9 (13·2)
Duration of employment (y)	8·9 (7·8)	8·5 (7·4)

Table 2 shows the mean and standard deviation (SD) for the serum bile acid measurements in the four HCBD exposure groups. A clear dose-effect relation was found for DC, GDC, TCDC, and DCTOT. These measurements had a significant positive log-linear relation with exposure to HCBD, controlling for age and exposure to solvent.

Table 3 shows the mean (SD) for the serum bile acid measurements in the two SOLVENT exposure groups. CDC, TC, and CATOT had a significant negative relation with solvent exposure, controlling for age and exposure to HCBD.

Other serum bile acid measures and all liver function tests showed no significant relations with exposure to either HCBD or SOLVENT. Neither age nor duration of employment was significantly related to any of the measures of serum bile acids.

STUDY B

Table 4 shows the mean age, duration of employment, and alcohol intake of subjects. Exposed subjects were older, had worked in the factory for slightly longer, and drank less alcohol than unexposed subjects. Four subjects took some form of regular medication (all in exposed group: anti-hypertensive, anti-inflammatory, thyroid hormone) and none reported any history of liver disease.

Table 5 shows the mean (SD) of plasma bile acid measurements for the two TCE exposure groups. Highly significant increases in the exposed group, controlling for age and intake of alcohol, were seen for GC, GDC, CDC, TC, TCDC, GTOT, TTOT, CATOT, CDCTOT, and BILTOT. Controlling for use of medication had only a minimal effect on these differences.

No significant relation was seen between other bile acid measures or any of the tests of liver function and exposure whether or not possible confounding factors were controlled. Age, duration of employment, and intake of alcohol were not significantly related to any of the bile acid measures.

No significant relations were found between any bile acid measure and serum or plasma cholesterol concentration in either study.

Discussion

Study A showed a clear positive relation between four serum bile acid measures (GC, DC, TC, and DCTOT) and exposure to HCBD, without associated changes in liver function tests. Some serum bile acid measures (CDC, TC, and, CATOT) were significantly lower in the group of workers exposed to a higher SOLVENT concentration compared with workers with a lower exposure. Again, no associated changes in liver function tests occurred. Workers in the second highest HCBD exposure group were the same as those in the highest

Table 2 Fasting serum bile acid concentrations (μmol/ml) in workers by exposure to hexachlorobutadiene

	Hexachlorobutadiene exposure (ppm)			
Bile acid	*0 (n=7) Mean (SD)*	*0·005 (n=15) Mean (SD)*	*0·01 (n=8) Mean (SD)*	*0·02 (n=5) Mean (SD)*
GC	0·86 (0·72)	0·30 (0·35)	0·21 (0·32)	0·78 (0·79)
GCDC	1·68 (1·18)	1·13 (0·91)	1·44 (1·62)	3·16 (2·41)
GDC**	0·36 (0·30)	0·86 (0·42)	1·03 (0·80)	1·89 (1·86)
GLC	0·39 (0·35)	0·22 (0·25)	0·26 (0·15)	0·22 (0·15)
CA	1·15 (0·94)	0·85 (1·28)	0·30 (0·45)	0·59 (0·57)
CDC	0·61 (0·37)	0·97 (1·07)	0·30 (0·24)	0·69 (0·52)
DC***	0·79 (0·43)	0·98 (0·55)	1·33 (0·90)	2·17 (0·75)
UDC	0·24 (0·24)	0·60 (0·36)	0·34 (0·17)	0·49 (0·16)
TC	0·40 (0·34)	0·51 (0·26)	0·20 (0·09)	0·27 (0·21)
TCDC*	0·39 (0·35)	0·43 (0·25)	0·52 (0·41)	0·58 (0·22)
TDC	0·12 (0·21)	0·19 (0·21)	0·27 (0·24)	0·25 (0·24)
TUDC	0·13 (0·16)	0·22 (0·12)	0·11 (0·12)	0·21 (0·07)
GTOT	3·28 (1·81)	2·51 (1·35)	2·94 (2·64)	6·05 (5·06)
TTOT	1·03 (0·93)	1·35 (0·54)	1·10 (0·72)	1·30 (0·72)
CATOT	2·40 (1·17)	1·67 (1·51)	0·71 (0·54)	1·64 (1·09)
CDCTOT	2·69 (1·50)	2·54 (2·03)	2·26 (1·95)	4·43 (2·51)
DCTOT****	1·25 (0·66)	2·02 (0·99)	2·64 (1·21)	4·31 (2·28)
BILTOT	7·10 (2·77)	7·26 (4·15)	6·31 (3·49)	11·30 (5·67)

*p < 0·05; **p < 0·03; ***p < 0·01; ****p < 0·001, probability of zero regression coefficient, controlling for age and exposure to solvent.

Table 3 Fasting serum bile acid concentrations (μmol/ml) in workers by exposure to solvents

Bile acid	Solvents: Lower exposure (n=27) Mean (SD)	Solvents: Higher exposure (n=8) Mean (SD)
GC	0·54 (0·59)	0·21 (0·31)
GCDC	1·65 (1·50)	1·44 (1·62)
GDC	0·92 (0·96)	1·03 (0·80)
GLC	0·26 (0·27)	0·26 (0·15)
CA	0·88 (1·08)	0·30 (0·45)
CDC*	0·83 (0·85)	0·30 (0·24)
DC	1·15 (0·74)	1·33 (0·90)
UDC	0·48 (0·37)	0·34 (0·17)
TC**	0·44 (0·28)	0·20 (0·09)
TCDC	0·45 (0·27)	0·52 (0·41)
TDC	0·18 (0·21)	0·27 (0·24)
TUDC	0·19 (0·12)	0·11 (0·12)
GTOT	3·37 (2·73)	2·94 (2·64)
TTOT	1·26 (0·68)	1·10 (0·72)
CATOT*	1·85 (1·36)	0·71 (0·54)
CDCTOT	2·93 (2·06)	2·26 (1·95)
DCTOT	2·25 (1·60)	2·64 (1·21)
BILTOT	7·97 (4·31)	6·31 (3·49)

*p < 0·05; **p < 0·03; probability of differences between mean serum bile acid concentrations, controlling for age and exposure to hexachlorobutadiene.

Table 5 Fasting plasma bile acid concentrations (μmol/ml) in workers by exposure to trichloroethylene

Bile acid	Trichloroethylene: Unexposed (n=6) Mean (SD)	Trichloroethylene: Exposed (n=16) Mean (SD)
GC****	0·14 (0·18)	0·57 (0·28)
GCDC**	0·52 (0·39)	1·23 (0·97)
GDC	0·29 (0·22)	0·45 (0·37)
GLC	0·23 (0·11)	0·25 (0·30)
CA	0·42 (0·47)	0·41 (0·46)
CDC**	0·28 (0·19)	0·61 (0·48)
DC	0·46 (0·16)	0·67 (0·62)
UDC	0·11 (0·12)	0·35 (0·54)
TC***	0·00 (0·00)	0·19 (0·22)
TCDC**	0·00 (0·00)	0·25 (0·32)
TDC	0·00 (0·00)	0·04 (0·11)
TUDC	0·00 (0·00)	0·02 (0·04)
GTOT**	1·17 (0·60)	2·49 (1·23)
TTOT**	0·00 (0·00)	0·48 (0·57)
CATOT**	0·56 (0·63)	1·16 (0·80)
CDCTOT***	0·79 (0·52)	2·09 (1·50)
DCTOT	0·75 (0·20)	1·16 (0·88)
BILTOT***	2·43 (1·29)	5·03 (2·45)

**p < 0·01; ***p < 0·001; ****p < 0·0001, probability of differences between mean plasma bile acid concentration, controlling for age and alcohol intake.

SOLVENT exposure group but the analysis takes into account any possible confounding between the two. It was not possible to determine whether any interaction occurred between the two exposures.

Exposure assessments for study A were based on environmental measurements taken on a number of occasions before this study. They therefore do not have the individual information obtainable from personal monitoring and do not take into account brief peak exposures. Nevertheless exposure to HCBD was considered to be well characterised quantitatively. SOLVENT exposures were comparatively lower (relative to their TLVs) and were not as well characterised.

Study A lacked direct data on previous hepatic disease and intake of alcohol, possible confounders of the exposure-serum bile acid (or liver function test) association. Intake of alcohol is not known to affect serum bile acid concentrations and was not found to have an important effect on plasma bile acid concentrations in study B. Raised serum bile acid concentrations have been found, however, in cases of established alcoholic liver disease,[11] and GGT is known to be an indirect marker of intake of alcohol.[12] The similar spread of abnormal GGT values between exposure groups, therefore, suggests that intake of alcohol was not appreciably different between exposure groups and that alcohol did not have an important confounding effect in this case.

Table 4 Age, duration of employment, and daily alcohol intake of workers in study B

	Unexposed (n=6) Mean (SD)	Exposed (n=16) Mean (SD)
Age (y)	32·5 (7·4)	46·4 (13·5)
Duration of employment (y)	13·0 (6·6)	17·0 (12·1)
Alcohol intake (g/day)	25·0 (38·7)	13·1 (28·2)

Study B showed highly significant increases in a range of individual and summed bile acids in workers exposed to TCE. No measures were significantly decreased and results of liver function tests did not differ between exposed workers and controls.

Assessment of exposure was based primarily on an inspection of work duties. Exposure was easily dichotomised and this was consistent with previous personal monitoring. Allocation to exposure groups is therefore likely to have little error.

Study B had detailed information on most potential confounders of importance. None of these were significantly related to any of the bile acid measures. These results strongly suggest that the TCE-bile acid relations found in this study are real.

It has been suggested that serum cholesterol concentration may be related to serum bile acid concentrations because of the role of cholesterol as a precursor in bile acid synthesis.[13] No such relation was found in either of our studies.

The positive exposure-bile acid relations seen for HCBD and TCE are consistent with a number of previous studies of workers exposed to one or more organic solvents.[5-7] Decreases in CDC, TC, and CATOT in SOLVENT exposed workers were

found. Other studies that found increased serum bile acid concentrations almost certainly involved solvent exposures substantially higher than those found in this study. So the lack of a clear positive serum bile acid-solvent effect in this case would not necessarily be inconsistent with these other studies. Possible confounding factors with SOLVENT exposures were not well characterised in study A so the potential for bias is higher than for exposure to HCBD and TCE. The potential for spurious results arising from multiple testing should also be considered for all three exposures. The negative SOLVENT-serum bile acid relations found must therefore remain open to question.

Different mean bile acid concentrations were found for unexposed workers in the two studies. Also the exposed workers in study B had lower bile acid concentrations for most measures compared with the unexposed workers in study A. This suggests that either the populations in the two studies really have different bile acid concentrations or some difference existed in the sensitivity of the analytical technique. As the technique was standard for both studies a possible explanation is the use of heparinised tubes (and thus the determination of *plasma* bile acid concentrations) in study B compared with tubes without anticoagulant (and the determination of *serum* bile acid concentrations) in study A. This should not affect the relative differences between exposure groups within each study.

The further finding of no exposure-liver function test response for any of the exposures is also consistent with previous studies that have found changes in serum bile acid concentrations apparently in response to certain exposures without accompanying changes in standard liver function tests.[5-7] These findings support the suggestion that serum bile acids are a more sensitive indicator of changes in liver function than the standard liver function tests.[4,6] Most standard tests of liver function actually reflect hepatocyte integrity rather than changes in hepatocyte function.[14] They may therefore be less sensitive to changes in hepatocyte function that are not accompanied by changes in hepatocyte membrane permeability. As there was no other objective measure of hepatocyte function in this study, however, actual sensitivity of serum bile acid concentrations as a measure of change in liver function cannot be determined absolutely. Nevertheless, it would seem likely that changes in bile acid concentrations reflect a change in liver function. It is the significance and reversibility that remain to be determined.

Consistent with the increases in plasma bile acid concentrations in workers exposed to TCE, serum bile acids have been shown to increase in a dose dependent manner in rats exposed to TCE, with increases occurring at concentrations where no increase in the liver enzyme activities assayed (ALT and ALP) occurred.[8] A recent in vitro study has shown reversible inhibition of rat hepatocyte transport mechanisms by chlorinated hydrocarbon solvents, associated with a decrease in ATP concentrations and ATPase activity.[15] These studies suggest that raised serum bile acid concentrations in workers exposed to solvents may be the result of inhibition of hepatocyte transport mechanisms by solvent molecules. Other authors have suggested this and also other mechanisms as discussed in a recent review by Franco.[16] Interestingly, this review also indicated that no rise in serum bile acid concentrations occurred in workers exposed to chlorinated solvents. Our data, both in this report and from other studies in experimental animals, indicate that some chlorinated solvents do cause increases in serum bile acid concentrations.

In conclusion, our study found a dose dependent increase in concentrations of some serum bile acids with increased exposure to HCBD and highly significant increases in a range of plasma bile acid measures in workers exposed to TCE. Some serum bile acid concentrations decreased in workers exposed to a solvent mixture, but the exposures were low and not well characterised. No exposures were associated with changes in liver function tests. These results support the suggestion that chlorinated solvents can be associated with raised serum bile acid concentrations and that serum bile acid concentrations are more sensitive indicators of changes in hepatic function than liver function tests.

The results from these two studies are best viewed as preliminary indicators of the possible hepatic effects of chlorinated hydrocarbons at concentrations not in excess of current workplace exposures. They also indicate the potential use of monitoring one or more individual or summed serum bile acid measures in workers exposed to possible hepatotoxicants. A more complete study is now needed with larger numbers of subjects, environmental and biological measures of exposures, and thorough measurement of potential confounders. These results should then be compared with animal studies, in which a range of exposures, administered by inhalation and checked by blood concentrations, could be investigated.

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