

TECHNICAL REPORT FOR "SURVEILLANCE METHODS FOR
SOLVENT-RELATED HEPATOTOXICITY" (SERCA 1 K01 OH00165-01)

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The investigation "Surveillance Methods for Solvent-Related Hepatotoxicity" (SERCA 5 K01 OH00165-03) was completed in September 2001. The following Technical Report is submitted in advance of publications/manuscripts which are currently under development.

The purpose of this investigation was to address the following Study Aims:

- 1) Determining if changes in hepatic parenchymal echogenicity--in relation to routine hepatic transaminase levels--occur in solvent exposed painters compared to a referent group of less exposed carpenters.
- 2) Determining whether elevations in pro-collagen biomarkers of fibrosis and fibrogenesis occur in painters compared to referents.
- 3) Determining whether elevations in serum bile acids occur in painters compared with the referent group, and
- 4) Determining whether a dose-response relationship exists between hepatic surveillance end-points and cumulative or subacute exposure to solvents.

Subject population:

The study population included the following groups of workers, representing a broad spectrum of organic solvent exposure in the course of usual occupational activities: 1) industrial painters, representing a generally high mixed solvent exposure group; 2) millwrights, representing an intermediate exposure group; and 3) carpenters, representing a minimally exposed referent group.

Procedures:

Data collection included an interview-administered questionnaire to determine cumulative career solvent exposures (by means of a semi-quantitative cumulative exposure index), with both venous sample collection and hepatic ultrasonography performed at Kadlec Medical Center (Richland, WA). Laboratory evaluation of blood samples included tests of hepatic function parameters, specifically cytokine analyses performed at the Fred Hutchinson Cancer Research Center (Dr. George McDonald; pro-collagen biomarkers of hepatic fibrosis); and serum bile acids at the Veterans' Administration Hospital (Dr. Sum P. Lee).

Hard-copy hepatic sonograms were interpreted for qualitative radiographic changes (Drs. Wang, Carpenter, and Dubinsky) and quantitative sonographic measurements with referent phantom images (David Green).

Statistical Analyses:

In addition to comparisons of hepatic biomarkers by job classification (carpenter, millwright, painter), multiple linear regression analyses were performed to assess hepatic biomarker

levels as a function of a semi-quantitative cumulative exposure index, adjusting for confounders of age, gender, alcohol intake, body mass index, and serologic evidence of prior Hepatitis B/C infection.

Significant Findings:

A total of 102 participants completed their data collection sessions at Kadlec Medical Center, as described above (see Procedures). **The study results are provided with a Table of Contents/List of Tables below, with a summary abstract.** Study subjects have been sent copies of their routine laboratory results, with a cover explanatory letter, per Human Subjects protocol, using approved Explanatory Letter Form.

A significant elevation in the hepatic cholestatic enzyme gamma glutamyl transpeptidase (GGT) was observed in painters, with a mean level of 41 IU/L, compared with carpenters and millwrights 27 IU/L ($p \leq 0.05$). This effect was also demonstrated by a significant exposure-response for GGT and cumulative career exposure to mixed general solvents, observed in multiple linear regression analyses controlling for age, gender, alcohol, body mass index ($p \leq 0.05$)

In summary, the findings confirmed the utility of gamma-glutamyl transpeptidase (GGT), a biochemical marker for hepatic cholestasis, in assessing early hepatic changes in solvent-exposed workers. Evidence for a significant exposure-response relationship was identified, after controlling for the effects of age, alcohol intake, body mass index, and serologic findings of Hepatitis B surface antigen or anti-Hepatitis C antibody. In contrast, no significant or consistent associations have been identified thus far between solvent exposure and hepatic transaminase levels (ALT,AST), serum bile acid levels, or hepatic cytokine levels with the exception of a trend towards elevation of Procollagen III observed in millwrights ($p=0.07$).

Qualitative ultrasound readings (with 3 blinded radiologists) demonstrated a trend for moderate to severe parenchymal changes in association with cumulative career exposure to general solvents ($p=0.07$), with a significant association between moderate-severe sonographic changes and cumulative general solvent exposure index values greater than the median ($p=0.03$). Use of a hepatic "phantom" for quantitative ultrasonographic readings demonstrated no exposure-response association, and appears to offer little utility in surveillance for early hepatic changes.

Usefulness of Findings:

The significant association between changes in routinely available tests of hepatic function, namely the cholestatic hepatic

enzyme GGT, as well as ultrasonography, in relation to mixed general solvent exposure, demonstrates potential efficacy for these tests in population surveillance of solvent-exposed workers. The current findings do not suggest an efficacious role for serum bile acid or cytokine biomarkers for detecting early hepatic injury related to solvents, though the finding of elevated Procollagen III levels in millwrights bears further investigation.

A specific assessment of utility for these tests will be provided in subsequent manuscripts that will be submitted for publication.

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ABSTRACT

SURVEILLANCE METHODS FOR SOLVENT-RELATED HEPATOTOXICITY

Purpose:

The purpose of this investigation was to determine the efficacy of clinically available tests of hepatic function in detecting early hepatic changes associated with mixed solvent exposures.

Methods:

A cross-sectional investigation of 102 workers with a range of cumulative career exposure to organic solvents was conducted, ranging from carpenters with low cumulative exposure, millwrights with intermediate exposure, and industrial painters with higher cumulative exposure to mixed organic solvents.

Data collection included an interview-administered questionnaire used to determine a cumulative exposure index to mixed general solvents, with both venous sample collection and hepatic ultrasonography performed at a regional medical center (Richland, WA). Laboratory evaluation of blood samples included tests of hepatic function parameters (ALT, AST, AP, GGT, and bilirubin), serum bile acid levels, and cytokine (pro-collagen biomarkers of hepatic fibrosis) analyses. In addition to comparison of hepatic biomarkers by job classification (carpenter, millwright, painter), multiple linear regression analyses were performed to assess hepatic biomarker levels as a function of a cumulative exposure (by a semi-quantitative exposure index), adjusting for confounders of age, gender, alcohol intake, body mass index, and serologic evidence of prior Hepatitis B/C infection.

Results:

A significant increase in the hepatic cholestatic enzyme gamma glutamyl transpeptidase (GGT) was observed in painters, with a mean level of 41 IU/L, compared with carpenters and millwrights (27 IU/L; $p \leq 0.05$). This effect was also demonstrated by a significant exposure-response for GGT and cumulative career exposure to mixed general solvents, observed in multiple linear regression analyses controlling for age, gender, alcohol, body mass index ($p \leq 0.05$). In association with this cholestatic biochemical change, a trend towards moderate to severe sonographic parenchymal changes in association with general solvent exposure was observed by ultrasound ($p=0.07$).

Other tests for hepatic biochemical function including hepatic transaminases (ALT, AST) and serum bile acids demonstrated no consistent exposure-response relationship with mixed solvents. Among the pro-collagen biomarkers of fibrosis, a trend towards

elevation of Procollagen III was observed in millwrights ($p=0.07$).

Conclusion:

The significant exposure-response relationship between career cumulative exposure to mixed solvents and hepatic GGT levels supports a primary cholestatic effect of solvents, with an associated trend for hepatic parenchymal changes on ultrasonography. The absence of consistent elevations in cytokine biomarkers of fibrosis suggests that these ultrasonographic findings represent steatosis rather than fibrosis. The cholestatic hepatic effects observed in this study appear to be most prominent in industrial painters, a group with high exposure to mixed solvents.