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.

Acknowledgments:

The current research was funded through a SERCA Award provided by NIOSH (5 K01 OH00165-03). Dr. Brodkin worked closely with the International Brotherhood of Painters and Allied Trades (IBPAT), Local Unions 1789 (Richland, WA) and 427 (Pasco, WA) to identify painters; Millwrights' (Local Union 1699 to identify millwrights; and the United Brotherhood of Carpenters (Pacific Northwest District Council/Local 1829), and Carpenters' Hanford Local 2403 to identify carpenters.

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.

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TABLE 1 DEMOGRAPHICS BY EXPOSURE GROUP, ALL SUBJECTS

	Carpenter	SD	Millwright	SD	Painter	SD	Significance, Oneway ANOVA
N	44		24		34		
Age	47	8	50	9	43	8	.006 +
Gender, male	42	95	24	100	32	94%	Chi2 = 1.4 P= 0.50
Female	2	5			2	6%	
Height	70	2	70	2	70	3	.53
Weight	198	27	206	30	193	31	.26
BMI (kg/m^2)	28	4	29	4	28	4	.47
Ethnicity (check values)							chi2=10.1 P= 0.43
1	1	2					
2					1	3%	
3	39	93	24	100	31	91%	
4					2	6%	
5	1	2					
6	1	2					
Education	12	1	12	1	11	1	chi2 P= 0.17 (n c=34, m=17, p=)33
Duration of employment (years in trade)	22	7	22	9	20	7	0.42
Alcohol consumption (g/week)							
Current (last 6 months)	58	93	88	110	68	92	chi2 P= 0.94
Maximum (any 6 months)	148	134	224	191	170	165	chi2 P= 0.18

+ test output below

Hetrogeneity of Gender, ethnicity, education, current alch, max alch across of job was assessed with chi2 because of non-normal distributions.

All non-normal distributions were also assessed for differences across levels of job with the Median test (Fishers exact). None were significantly different.

TABLE 2 BIOCHEMICAL DATA BY JOB CATEGORY

	Carpenter	[Millwright + Painter]	Millwright	Painter	Significance chi2 [Millwright + Painter]	Significance chi2 Carp, Mill, Paint
N	44	58	24	34		
HepC+	**1 (2%)**		**0**	**2 (6%)**		
Total bilirubin	0.6 (0.3)	0.6 (0.3)	0.6 (0.3)	0.6 (0.3)	0.53 (RS .85)	0.69
Direct bilirubin	0.2 (0.1)	0.2 (0.1)	0.2 (0.1)	0.1 (0.1)	0.72 (RS .79)	0.70
ALT	28 (15)	29 (15)	27 (12)	31 (16)	0.82 (RS .50)	0.85
AST	29 (7)	19 (10)	27 (9)	31 (10)	0.41 (RS .47)	0.27
GGT	27 (16)	35 (25)	27 (13)	41 (30)	0.24 (.11 trend test) (**.05** Rank Sum test*)	0.095 *
Alk Phos	70 (22)	73 (17)	72 (17)	74 (18)	0.39 (RS .24)	0.80
H6Ai C (shbaic, hemaglobin, diabetes)	5 (0.5)	5 (0.3)	5 (0.3)	5 (0.5)	0.38 (RS .62)	0.58 n=98 c=41, m=23, p=34

GGT BY JOB CATEGORY

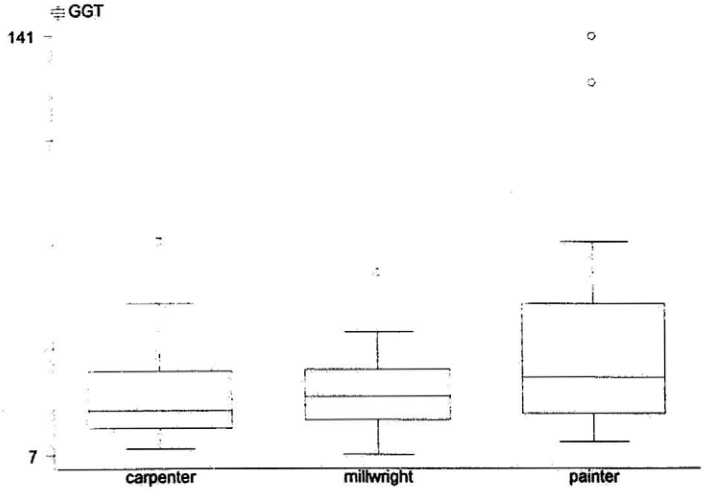

TABLE 3 SERUM BILE ACIDS, OUTCOME CHANGES BY CATEGORICAL EXPOSURE

Serum Bile Acid data ID #52 is missing all of this data from file smhsys1.dta. Is this correct?

Mean (SD)	Carpenter	[Millwright + Painter]	Millwright	Painter	Mann-Whitney rank sum test [Millwright + Painter]	Median Test fishers exact carp, mill, painter
N	44	57	24	33	P-value	P-value
Cholic acid	21 (29)	18 (18)	16 (15)	20 (20)	.92	.79
Chenodeoxycholic, cheno	23 (16)	28 (35)	18 (10)	34 (44)	.87	.42
Tauroiholais, tc	68 (116)	44 (27)	45 (36)	44 (20)	.99	.79
Glycdoholaz, gc	43 (29)	41 (20)	40 (25)	42 (17)	.63	.28
Glycodeoxycholate, gcdc	61 (48)	68 (41)	68 (50)	68 (34)	.14	.46
Glycodeoxycholate, gdc	33 (27)	31 (33)	42 (43)	24 (19)	.47	.34
Taurolithocholate, tlc	1.9 (3.0)	1.1 (2.2)	0.6 (2.0)	1.4 (2.3)	.12	.11
Glycolithoiholate, glc	12 (11)	12 (11)	12 (11)	13 (11)	.97	.93

TABLE 4 CYTOKINE AND PROCOLLAGEN LEVELS

	Carpenter	[Millwright + Painter]	Millwright	Painter	Mann-Whitney U [Millwright + Painter]	Median Test fishers exact Carp,mill,paint
					P-value	P-value
Tgfb1	8.8 (5.5)	7.7 (5.4)	6.6 (3.6)	8.5 (6.5)	.33	.65
ProcollageIII (PIIIP)	.74 (.17)	.76 (.14)	.82 (.14)	.72 (.13)	.33	**.07**
TNF-Alpha	348 (1062)	296 (769)	498 (1088)	153 (380)	.68	.36
TNF-RI	615 (177)	607 (178)	641 (211)	583 (149)	.93	.79

PROCOLLAGEN III LEVELS BY JOB CATEGORY

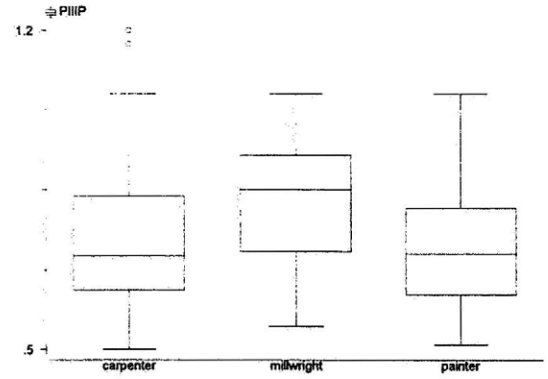

TABLE 4B QUALATATIVE ULTRASOUND READINGS BY EXPOSURE INDEX

	n with Zero expousre	N	Normal	Mild	Moderate-Severe	Any	Mantel-Haenszes test of trend, p-value
General Solvent	15						
Above median (12.9)		51	19 (37)	20 (39)	12 (24)	32 (63)	.07*
Below median		51	24 (47)	23 (45)	4 (8)	27 (53)	
Metal Exposure (0.2)	48						
Above median		54	21 (39)	23 (43)	10 (19)	33 (61)	.36
Below median		48	22 (46)	20 (42)	6 (13)	26 (54)	
Solvent alone (4.1)	29						
Above median		51	20 (39)	22 (43)	9 (18)	31 (61)	.49
Below median		51	23 (45)	21 (41)	7 (14)	28 (55)	
Chlorinated Solvent (0.0)	77						
Above median		25	11 (44)	10 (40)	4 (16)	14 (56)	.90
Below median		77	32 (42)	33 (43)	12 (16)	45 (58)	

*p=0.03 for comparison of moderate-severe vs. normal and mild

TABLE 5 QUANTITATIVE ULTRASOUND (PHANTOM) BY JOB CATEGORY AND VARIOUS SEMI-QUANTITATIVE SOLVENT EXPOSURE INDICES

	Median		Chi^2	90th percentile		
	Low	High	P-value	Low	High	P-value
Carpenter (controls, n(%))	23 (52)	21 (48)		41 (93)	3 (7)	-
Millwright + Painter	29 (50)	29 (50)	.82	53 (91)	5 (9)	.74
Millwright	10 (42)	14 (58)	.40	21 (88)	3 (13)	.43
Painter	19 (56)	15 (44)	.75	32 (94)	2 (6)	.87
Metal Exposure						
Above median	23 (48)	25 (52)		43 (90)	5 (10)	
Below median	29 (54)	25 (46)	.56	51 (94)	3 (6)	.36
Solvent alone *						
Above median	26 (51)	25 (49)		47(92)	4 (8)	
Below median	26 (51)	25 (49)	1.0	47 (92)	4 (8)	1.0
Chlorinated Solvent						
Above median	38 (49)	39 (57)		70 (91)	7 (9)	
Below median	14 (56)	11 (44)	.56	24 (96)	1 (4)	.41
Job 15 years						
Above median	10 (48)	11 (52)		20 (95)	1 (5)	
Below median	42 (52)	39 (48)	.73	74 (91)	7 (9)	.56

TABLE 6 MULTIPLE LINEAR REGRESSION MODELS, ADJUSTED DIFFERENCES IN HEPATIC BIOCHEMICAL FUNCTION BY GENERAL SOLVENT EXPOSURE INDEX

General Solvent	Age	Gender	BMI	Alcohol	HepC+		R^2	Index Coefficient (P)
AST	.04	2.88	.14	.01	19.08 ***		.101	.02 (.65)
ALT	-.10	9.32	.79*	.02	33***		.187	.10 (.13)
GGT	.12	12.1	.87	.01	4.17		.043	.22 **(.04)**
AP	-0.04	-19	.02	-.00	0.48		-.023	.03 (.78)
Direct Bilirubin	-.00	.04	-.00	-.00	-.03		-.018	-.00 (.41)
D/Total Bilirubin	.00	.11	-.02 **	-.00	-.19		.028	-.00 (.60)
H6Ai C (shbaic, hemaglobin, diabetes)	.01 **	.16	.02*	-.00	-.16		.11	-.00 (.79)

AST = B1 Age + B2 Gender + B3 BMI + B4 Alcohol + B5 Hep C Serology + B6 Packyears + B7 Solvent Exposure*

*Solvent exposure assessed by semi-quantitative index

*<0.05;**<0.01;***<0.001

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