Workers of the printing industry and hepatic damage


Key words: AST-ALT-ALP-γ-GT, solvents, VOCs (volatile organic compounds), typesetting industry

Parole chiave: AST-ALT-ALP-Gamma-GT, solventi, VOC (composti organici volatili), industria

Abstract

Background: Typesetting industry is still the primary instrument of communication, despite the development of new technological systems. This study focuses on the analysis of the hepatic effects induced by the use of some organic solvents employed in the printing industry.

Methods: We studied a group of 194 workers: 93 exposed and 101 not exposed. The level of the exposure to chemical pollutants were assessed through the environmental monitoring of blood concentrations and the analysis of airborne substances. The health survey was performed through the collection of the medical history and the use of hepatic tests, which were evaluated by calculating Mean, Standard Deviation, Student’s t-test and X² test with Yates Correction, to investigate statistically significant differences in some hepatic parameters: AST, ALT, ALP, GGT, fractional and total bilirubin. The environmental data sometimes exceeded the TLV-TWA.

Results: The clinical evaluation of the hepatic parameters showed statistically significant differences as to the hematic concentrations of AST, ALT, GGT.

Discussion: The results we obtained support the hypothesis of a risk among the printing industrial workers attributable to the hepatotoxic solvents. This risk seems to be related to the use of a mixture of solvents, although at low doses, and the analysis of the results obtained confirms the validity of the investigation for the health screening protocol adopted in order to identify subjects and/or population at risk of hepatotoxicity.

Introduction

The printing industry has always been considered a possible risk source, not only for the workers but also for the people (1), exposed also to the synergistic effects of environmental pollution (2-13).

The new technologies are in fact associating and replacing the traditional system of printing, causing the elimination or the drastic reduction of some well known risk agents (for example the inorganic lead) but they should be carefully inquired to point out new potential damages for workers (14-16).

Each printing process (printing, screen printing, ink jet, rotogravure, flexigraphic) is distinguished as for the characteristics of the matrices and for the solvents used, which are the real causes of hepatotoxic risks.
Print shop workers (or any other print service provider) are in contact with potentially hazardous solvents, and the right assessment of the chemical risk in the workplace is necessary in order to know the risks for the workers’ health.

The purpose of the study is to examine the potential effects on the liver due to the use of solvents in the printing industry.

**Methods**

**Study population**

140 subjects (A), exposed to solvents during their worktime and a control group of 156 subjects (B), not exposed to such chemicals were studied. The exposed subjects were selected from the staff involved in the productions requiring the use of chemical compounds with different potential hepatotoxicity.

These subjects were working for a total of seven hours a day for at least five days a week, rotating in different departments. The control group was composed of subjects not exposed to hepatotoxic substances, involved in the qualitative (selection) and quantitative (the counting) verification of the products, in the packaging and shipping of goods, in the preparation of rolls for printing.

We excluded from the study the workers who reported the following exclusion factors in the anamnestic questionnaire: excessive alcohol consumption (more than 2-3 alcoholic units/day for men and more than 1-2 alcoholic units/day for women, where 1 alcoholic unit corresponds to about 12 grams of ethanol) (17), smoking status (smoker: a person who smokes at least 100 cigarettes in his lifetime and who currently smokes every day or almost) (18); use of hepatotoxic drugs; liver diseases or other clinical signs of previous or current liver diseases; family history of liver diseases; non occupational exposure to solvents or other substances and hepatotoxic drugs. Workers with seniority of less than 2 years were also excluded, because liver effects take longer to be induced. The total number of exposed workers (group A) was 93. Out of a sample of 156 subjects we selected, as a control group, 101 employees in office work and not exposed to solvents or to the other risk factors. They were made comparable to the exposed workers as for age and seniority. The characteristics of the groups object of this study are reported in table 1.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Exposed</th>
<th>Not exposed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>93</td>
<td>101</td>
</tr>
<tr>
<td>Working Seniority (years)</td>
<td>15.4 ± 6.04</td>
<td>15.2 ± 5.90</td>
</tr>
<tr>
<td>Mean</td>
<td>15.4 §  ± 6.04</td>
<td>15.2 ± 5.90</td>
</tr>
<tr>
<td>min-max</td>
<td>1 - 21</td>
<td>1 - 20</td>
</tr>
<tr>
<td>Age (years)</td>
<td>46.07 ± 6.29</td>
<td>44.08 ± 7.47</td>
</tr>
<tr>
<td>Mean</td>
<td>46.07 § ± 6.29</td>
<td>44.08 ± 7.47</td>
</tr>
<tr>
<td>min-max</td>
<td>29 - 52</td>
<td>31 – 54</td>
</tr>
</tbody>
</table>

To assess the effect of the solvents present in the workplace on the liver function tests, we compared: mean and standard deviation of the blood levels of glutamic-oxaloacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), gamma-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), fractional and total bilirubin between the two groups.

We also checked the number of workers of both groups who had the liver test levels higher than the normal range. The resulting frequencies were compared.

The reference values of our laboratory were: GOT 10-34 IU, GPT 10-47 IU, GGT 10-52 IU, alkaline phosphatase 105-290 IU; Total Bilirubin: until 1.2 mg/dL; Fractional Bilirubin until 0.3 mg/dL.

All subjects consented to the processing of their personal data, admitting to be aware that these data belonged to the category of
“sensitive” data. All subjects also agreed that the data obtained were treated anonymously and collectively, with methods and scientific purposes in accordance with the principles of the Declaration of Helsinki.

Environmental survey

The Material Safety Data Sheets of the products used in the printing industry object of this study were revised. Through an environmental survey and the analysis of the airborne substances collected (both in form of vapor and particulate matter) we assessed the levels of the exposure to chemical agents (tab. 2).

<table>
<thead>
<tr>
<th>Tests compared</th>
<th>Exposed</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (got)</td>
<td>Mean 25.41</td>
<td>Mean 21.13</td>
</tr>
<tr>
<td>P= 0.0001</td>
<td>DS ± 8.04</td>
<td>DS ± 7.65</td>
</tr>
<tr>
<td>ALT (GPT)</td>
<td>Mean 26.84</td>
<td>Mean 21.19</td>
</tr>
<tr>
<td>P=0.0001</td>
<td>DS ± 11.56</td>
<td>DS ± 8.69</td>
</tr>
<tr>
<td>GGT</td>
<td>Mean 30.87</td>
<td>Mean 24.73</td>
</tr>
<tr>
<td>P=0.006</td>
<td>DS ± 14.37</td>
<td>DS ± 11.52</td>
</tr>
<tr>
<td>ALP</td>
<td>Mean 194.49</td>
<td>Mean 182</td>
</tr>
<tr>
<td>P=0.116</td>
<td>DS ± 51.84</td>
<td>DS ± 57.86</td>
</tr>
</tbody>
</table>

The methods and the sampling/analysis techniques used for the qualitative/quantitative evaluation of the chemical risk factors identified were the Zambelli mod. “Chronos” individual samplers, in some fitted with a suitable vial of activated carbon in order to perform the collection of samples at suction. The vials containing two different sections of activated carbon of granulometry from 20 to 40 mesh, were refrigerated for the next analytical phase after the absorption of the pollutants dispersed in the vapor phase.

In the laboratory the elution of vapors of the absorbed solvents and their gaschromophotograph dosage was performed adopting the following working conditions: 2 meter - 1/8 inch diameter chromatographic column, 1% SP 1000 on Carbovack C, 60-80 mesh; temperature 230° for oil, toluene, xylene, and solvent 20721, 130° for 1,1,1, - trichloroethane; FID detector.

The determination of the volatile organic solvents (“volatile organic substances”, VOC, or SOV in italian) was performed through some volumetric samplers placed 150 cm above the ground, with constant flow and tuned to 0.3-0.4 L/min, employing 20-40 mesh activated carbon as an absorption support. All determinations were performed for the employees assigned to the different operations on the basis of the levels of exposure and the sampling times of processing stages. Usually the sampling times coincided with the execution of the individual phases.

The methods of sampling and the analysis of solvent mixtures in the air consisted of the collection of activated charcoal on a special filter. The coal was then desorbed with carbon disulfide and analyzed by gaschromatography (NIOSH P & CAM-127). The exposure was assessed as the mean concentration for eight consecutive hours (TLV-TWA).

We used the method described by the American Conference of Governmental Industrial Hygienist (ACGIH) to calculate the TLV-solvent mixtures:

\[ \frac{C_1}{T_1} + \frac{C_2}{T_2} + \ldots + \frac{C_n}{T_n} \]

\( C = \) concentration of the substance in the atmosphere, \( T = \) TLV of the substance

If the sum of the previous fractions is higher than the unity, the exposure limit for the mixture of substances must be regarded as obsolete.

Health survey

For each of the 194 individuals selected we collected and evaluated:

a) Anamnestic data, with particular reference to: lifestyle (alcohol and smoking),
possible past or current clinical hepatic pathology, use of drugs, intake of hepatotoxic drugs and familiarity for hepatic diseases. The collection of these data focused on the identification of individual susceptibility to hepatotoxic drugs and of non occupational hepatobiliary pathologies;

b) Laboratory tests: AST/GOT, ALT/GPT, GGT, ALP (which are the most specific indexes of cytolysis and cholestasis), fractional and total bilirubin.

These hepatic tests were performed using 10 ml venous blood samples taken from each worker in the morning before eating. The blood samples were preserved in the workplace, in a refrigerator at + 4° until they were transferred to a lab, in a container and at the same temperature, and analyzed within three days. Data were analyzed to estimate the existence of a possible significant difference between the values of the different hepatic function tests observed in the group of exposed workers and in the control group.

The levels of serum-enzyme activity were assessed as follows:

- AST: method Nicotinamide Adenine Dinucleotide (NAD+) / Malate Dehydrogenase (MD+)
  - reference value in humans up to 10-38 IU/L;
- ALT: method NAD+/MD+ - reference value in humans up to 10-38 IU/L;
- GGT: enzymatic method - reference value in humans up to 0-53 IU/L;
- ALP: enzymatic method - reference value in adults 94-266 IU/L;
- Total bilirubin: reference values until 1.2 mg/L;
- Bilirubin fractional: reference values until 0.3 mg/L.

The data were also stratified in value ranges and their distribution in the two groups, A (exposed) and B (controls), was studied.

In both groups the subjects with altered tests were examined, and those with one or more modified tests were identified. We analyzed the distribution of the values of the altered tests, as for age and for working seniority, in the group of exposed and controls and compared with each other.

Statistical analysis

The statistical analysis of the data was based on the calculation of the mean, the standard deviation, the distribution and frequency in accordance with the nature of the individual variables. The differences between means were compared using the Student’s t-test for unpaired data. The differences between the frequencies of the individual variables were compared using the X²-test with Yates correction. When the values obtained showed p <0.05, the differences were considered significant. The data were processed using the program BMDP Solo ® Statistical Software.

Results

Results of the environmental survey

The examination of the tables of risk relative to the solvents and the inks, provided by the company, showed the presence of a conspicuous quantity of substances, some of which with defined hepatotoxicity (toluene, xylene, benzene, oil, 1,1,1, - trichloroethane, solvents 20721 isoparaffin) (19).

The concentrations of the solvent 20721 (isoparaffin> C10, aliphatic hydrocarbon) (TLV-TWA: 1000 mg/mc) ranged from a minimum of 46.8 mg/m³ to a maximum of 170.2 mg/m³, while the detected value for the 1,1,1, - trichloroethane ( TLV-TWA: 1910 mg/mc), during the washing of tub was 34.3 mg/m³.

The results of the analysis of the doses of the different solvents in chalcographic department pointed out that the values of workplace center were limited and relatively constant, varying from 13.6 mg/m³ to 20.9 mg/m³ for toluene and from 19.5 mg/m³ to
24.2 mg/m³ for xylene. The values for the machine operators were different reaching in some cases high levels: the variation of exposure was between 11.6 mg/m³ and 56.7 mg/m³ for toluene, not surpassing its TLV-TWA = 75.4 mg/m³, while the variation for the xylene which exceeded its TWA = 434 mg/m³ was between 74.1 mg/m³ and 552.6 mg/m³.

The VOCs in the workplace ranged from a minimum of 1.1 mg/m³ to a maximum of 20.5 mg/m³.

As to the TLV of mixtures, the sum of the ratios between the determinations of airborne substances and the related limits of exposure was higher than the unity for the detections made on the machine n° 4 operator in the rotogravure department (TLV mixture = 1.42) and on the machine n° 3 operator (TLV mixture = 1.13), while on the machine n° 2 operator (TLV mixture = 0.97) and on the machine n° 1 operator (TLV mixture = 0.57) it was lower than the unity. In all these situations, in particular for operator n° 3 and n° 4, a real condition of residual risk for exposure to hepatotoxic substances during the working activity appeared.

Results of health survey

The analysis of the results performed in the gravure and rotogravure departments showed a higher number of altered tests in the exposed (44 tests or 12%) compared with the controls (22 tests or 5%) with p > 0.05, and it showed a higher number of subjects with hepatic tests above the reference values, in the exposed (33 individuals or 36%) compared with the controls (15 individuals or 15%) with p <0.05:

In group A, 24 subjects or 26% showed one index of hepatic damage higher than the reference value, versus a 10% (10 subjects) in the control group (p = 0.06). 7 subjects or 8% in the exposed group had ALT and AST, above the reference values, versus 3 subjects or 3% in group B (p = 0.2). The alteration of all three indices of hepatic function was observed in 2 subjects or 2% of the two groups (p = 1).

The number of subjects with AST above the reference was higher among the exposed (7 subjects, or 10.2%) than among the controls (4 subjects, or 0.99%) and it showed a statistically significant difference in the comparison between the mean values of the two groups (exposed and controls) with p = 0.0001 (Table 2).

The number of subjects with ALT above the reference value was higher among the exposed (19 subjects, or 20.4%) than among the controls (9 subjects or 8.9%) and it showed a statistically significant difference in the comparison between the mean values of the two groups (exposed and controls) with p = 0.0001 (Table 2).

The number of subjects with levels of ALP above the reference was higher among the exposed (12 subjects or 12.9%) than among the controls (7 subjects or 6.9%), but there was no evidence of significant difference in the comparison between the mean values of the two groups with p = 0.116 (Table 2).

The number of subjects with levels of GGT above the reference was higher among the exposed (6 subjects, or 6.4%) than among the controls (2 subjects, or 1.98%), and also in this case the comparison between the mean values of the two groups was significant, with p = 0.006 (Table 2).

In both groups, no subject had levels of total and fractional bilirubin higher than the reference level, so there was no evidence of statistically significant difference in the comparison between the exposed group and the controls.

The analysis of the percentage of subjects in relation with ranges values of AST, ALT, and GGT is reported in table 3.

The differences for the values of bilirubin and alkaline phosphatase were not significant.

The comparison about the distribution of the values of normal and altered hepatic
function tests did not lead to statistically significant results with regard to the subdivision of classes for age and work seniority.

Discussion

The results suggest that the solvents used by the workers of the printing industry have an influence on the liver. In our research, the altered blood-chemistry hepatic parameters, in exposed vs not exposed subjects were ALT, AST, and GGT. Transferases, AST and ALT are the most frequently used indicators of hepatic cell necrosis. In particular the hepatocytes are the only cells that contain high levels of ALT; while AST is in the liver but also in other organs and apparatuses such as the heart, the skeletal muscle, the kidney and the brain (20).

In many studies increases in serum GGT are associated with the exposure to hepatotoxic chemicals (21-24).

In the absence of clinical signs the alterations found for AST and ALT suggest the existence of hepatic damage which may develop a mechanism of cytotoxicity against hepatocytes with the passage of enzymes into the blood stream. This situation allows to assume that the alleged risk factors for the liver may act through a predominantly non-cholestatic mechanism (25). The pathogenic mechanisms responsible for the functional and organic liver damage caused by solvents that we observed (26), could be the inflammation, the dysfunction of cytochrome P450, the mitochondrial dysfunction and the oxidative stress. The results obtained are in agreement with other research (27-32).

The significant differences we observed concerning hepatic enzymes ALT, AST and GGT among workers and controls acquire greater significance if we consider that:

a) no subject reported possible concentrations of AST/ALT and GGT due to alcohol consumption, previous hepatic diseases, intake of hepatotoxic drugs and use of solvents, paints or pesticides in his spare time or previous jobs;

b) the subjects were comparable for chronological age and working seniority.

c) the AST/ALT ratio was significantly higher in the group of exposed compared to the control group. This confirms that the damage induced by solvents is of cytotoxic nature (33).

The number of subjects with hepatic damage levels higher than the normal range (36%) for the exposed is in line with previous results on different categories of subjects exposed to hepatotoxic substances (34-38).

In the Italian population the persistent alteration of hepatic function tests is between 10% and 17% and the main etiological factors are alcohol abuse and viral hepatitis (39-41).

Table 3 - Percentage of subjects for value ranges of AST, ALT, GGT

<table>
<thead>
<tr>
<th>Variables</th>
<th>Exposed</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST UI/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 18</td>
<td>11.8%</td>
<td>35.6%</td>
</tr>
<tr>
<td>18-27</td>
<td>55.9%</td>
<td>50.5%</td>
</tr>
<tr>
<td>28-37</td>
<td>24.7%</td>
<td>6.9%</td>
</tr>
<tr>
<td>38-47</td>
<td>5.3%</td>
<td>2.9%</td>
</tr>
<tr>
<td>&gt;47</td>
<td>2.1%</td>
<td>0.99%</td>
</tr>
<tr>
<td>ALT UI/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25</td>
<td>51.6%</td>
<td>76.2%</td>
</tr>
<tr>
<td>25-34</td>
<td>28%</td>
<td>14.9%</td>
</tr>
<tr>
<td>&gt;34</td>
<td>20.4%</td>
<td>8.9%</td>
</tr>
<tr>
<td>GGT UI/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;33</td>
<td>62.3%</td>
<td>78.2%</td>
</tr>
<tr>
<td>33-52</td>
<td>31.1%</td>
<td>19.8%</td>
</tr>
<tr>
<td>&gt;52</td>
<td>6.4%</td>
<td>1.98%</td>
</tr>
</tbody>
</table>

Footnote a: = Statistically significant for X² test in exposed vs controls.
In another study the prevalence of non virus-correlated and non-alcohol-related hypertransaminasemia in the Italian population was 23% (42).

These data are significantly lower than the prevalence (36%) of hepatic markers above the normal range that we found in exposed workers.

According to the detected absence of non occupational risk factors and of other causes of modification of the studied tests, the results can confirm the hypothesis of hepatotoxicity by solvents present in the printing industry, as observed also in previous studies (43-46).

Lundberg et al. (47) estimated the hepatic damage (with significant increases in liver enzymes ALT, AST, GGT) induced in some industries (printing, chemicals, paints, glues, etc...) due to the exposure to solvents such as toluene, 1,1,1-trichloroethane, xylene.

**Conclusions**

The results we obtained are in line with those reported in the literature where the increase or alteration of the hepatic enzymes, not caused by infectious factors or alcohol consumption, is linked to the exposure to solvents in the workplace (48).

Considering that the solvents used in the working cycle have concentrations that vary, for the xylene, between 74.1 mg/m³ and 552.6 mg/m³ higher than the value of TLV/TWA 2011-2012, (434 mg/m³) for the airborne toluene between 11.6 mg/m³ and 56.7 mg/m³, below its own TLV/TWA (75.4 mg/m³) we conclude that:

a) The possible risk factors causing the enzymatic variation observed may also be caused by the association of several substances, although in low doses, of occupational origin (multideterminism and interaction).

b) The processes of distribution, of interaction with target organs, of metabolism and excretion of the chemical components of solvents, together with the toxic effects of their reaction products, are characterized by a high clinical and environmental variability (49).

c) The environmental results suggest that the toluene and the xylene are the solvents that more than others cause the increase of hepatic parameters (47, 50).

As to the results of the TLV mixtures, the sum of the ratios between the concentrations of airborne substances (toluene, xylene) and the corresponding exposure limit was higher than the unity as proved in a survey carried out in two cases in the rotogravure department. We believe this means that the risk of environmental pollution, for the mixtures of the organic solvents studied, is present, and that it increases the hepatotoxic effect.

**Riassunto**

**Lavoratori dell’industria tipografica e danno epatico**

**Introduzione:** L’industria tipografica è ancor oggi, la fonte primaria di comunicazione sebbene lo sviluppo dei nuovi sistemi tecnologici. L’oggetto di questo studio è l’analisi degli effetti epatici, indotti dall’utilizzo di alcuni solventi organici nei lavoratori dell’industria tipografica.

**Metodi:** Abbiamo studiato un gruppo di 194 lavoratori di cui 93 esposti e 101 non esposti. I livelli di esposizione ad inquinanti chimici sono stata valutata attraverso il monitoraggio ambientale con l’analisi delle sostanze aerodisperse. L’analisi sanitaria è stata svolta attraverso la raccolta dei dati anamnestici e l’utilizzo di test epatici, valutati attraverso il calcolo della Media, Deviazione Standard, Student T-test e X² test con correzione di Yates, per determinare statisticamente differenze significative in alcuni parametri epatici: AST, ALT, ALP, GGT, bilirubina frazionata e totale. I dati ambientali a volte superavano i TLV-TWA.

**Risultati:** La valutazione clinica dei parametri epatici, ha dimostrato differenze statisticamente significative come le concentrazioni ematiche di AST, ALT, GGT.

**Discussione:** I risultati ottenuti confermano l’ipotesi di un rischio epatotossico dovuto a solventi tra i lavoratori dell’industria tipografica. Questo rischio sembra essere legato all’utilizzo di miscele di solventi, sebbene a basse dosi e l’analisi dei risultati ottenuti conferma la validità dell’indagine per il protocollo sanitario adottato per identificare soggetti e/o popolazione a rischio di epatotoxicità.
References


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