Effects of Low-Dose Solvents on the Blood of Art Restorers

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Abstract: Effects on the Blood among Art Restorers: Francesco TOMEI, *et al.* Department of Occupational Medicine, University of Rome "La Sapienza"—Restoration of art works is usually assessed from an artistic viewpoint, and little attention is paid to the restorers' health. There appear to be no specific published reports. Art restoration is a craftsman's job, often done by students as part of their art courses, and they are exposed to low doses of a hard-to-classify range of different substances. This study employed a protocol designed for use in the workplace to check whether art restoration work had effects on the blood of the young people doing it. We studied 65 art restorer students and teachers, most of them young, who did different types of restoration work: on stone and archaeological findings, frescoes, paintings on wood and canvas, paper and parchment, plaster, fabrics, metals, wood or painted linings. Seventy-one non-exposed workers were compared as controls. We found significant reductions in RBC, Hb and Ht among exposed workers but no change in WBC. These findings suggest there may be problems in the effect on the blood of art restorers exposed to low doses of solvents. It would appear useful for these workers to undergo periodic blood screening tests, and the protocol we employed appeared suitable for identifying subjects at risk in their workplace.

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Key words: Art restoration, Solvents, Multiple exposure, Low dose, Blood effects

Art restoration is usually evaluated from an artistic viewpoint, and questions of the restorers' health receive scant attention. No significant paper has been published on art restorers but even a quick glance at this sector, from another angle, immediately reveals the large number of chemicals, mainly solvents, routinely employed. This is a craftsman's

job involving various degrees of discontinuous exposure to a variety of solvents, used in different ways for the various types and methods of restoration.

The potential toxic effects of solvents and their mixtures on the blood have been studied at high doses¹⁻⁴⁾ and even at low doses⁵⁻⁸⁾. Studies on other categories of workers found a correlation between exposure to solvents and changes in the blood. *Ørbaek et al.*¹⁾ studied workers in the paint-making industry exposed to organic solvents: they showed a general tendency to a low RBC count. In a group of painters and mechanics exposed to low doses of solvents, *Beving et al.*⁵⁾ found a reduction in the RBC count and an increase in the mean red cell volume but no real differences between exposed subjects and controls in the platelet count or volume.

Considering that young people may be more susceptible to effects on the blood, and therefore should be safeguarded more carefully, we studied art students and teachers, most of them young, in an art restoration institution. We therefore used an easy-to-administer protocol designed for use in the workplace to assess whether art restoration could lead to effects on the blood independently of effects on the liver of young people doing this work.

Materials and Methods

We studied 65 art restorers, students and teachers of both sexes (mean age 29.1 yr, SD 8.3, range 20-58), with 3.0 ± 1.5 years of work experience. There were 50 women, (mean age 26.8 ± 5.0 yr range 20-42) and 15 men (mean age 36.9 ± 11.9 yr, range 24-58). They alternate theoretical lessons during the week with practical sessions in the workshop, taking part in all types of restoration work: on stone and archaeological findings, frescoes, paintings on wood and canvas, paper and parchment, plaster, fabrics, metals, wood or painted linings.

The main solvents used in these jobs are ethanol, amyl-acetate, acetone, ammonia, hydrogen peroxide, butylamine, diacetone alcohol, dimethyl-formamide,

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oil and spirits of turpentine, petroleum, paraffin ethers, toluol, xylol, trichloroethane, trichloroethylene, polyvinyl acetate, aniline carbon tetrachloride, formaldehyde, phenols, methanol, nitro-diluents, mixtures of benzenic hydrocarbons, ethylene-chloride, methylchloride and methyl bromide.

In the presence of a physician, a targeted clinical history questionnaire was completed and each subject underwent a general clinical examination. The questionnaire was also focused on identifying the confounding factors such as pathologies involving changes in the blood, alcohol consumption, smoking habit, hemotoxic and hepatotoxic drugs assumption and history of liver diseases. The following laboratory tests were done: white blood cell count (WBC) with differential formula, red blood cells count (RBC), platelets count (PLT), hemoglobin concentration (Hb), hematocrit (Hct). To assess the general health of the subjects, in consideration of the fact that it has been suggested that low dose exposure to solvents may affect the liver⁹⁻¹³ the following hepatic tests were done: total and conjugated bilirubin, serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), cholinesterase, γ -glutamyltransferase (γ -GT) and alkaline phosphatase (AP). Reference values for all the tests were those commonly used by our laboratory: WBC 4,800–10,800/mm³; RBC males 470–610 $\times 10^4$ /mm³, females 420–540 $\times 10^4$ /mm³; PLT 130–400 $\times 10^3$ /mm³; Hb males 14–18 g/dl, females 12–16 g/dl, Hct males 42–52%, females 37–47%; total bilirubin <1 mg/dl; conjugated bilirubin <0.25 mg/dl; AST <46 IU; ALT <49 IU; γ -GT males <55 IU, females <50 IU; AP <290 IU.

We assessed the biological indicators for certain solvents, for which reliable, routinely employed methods are available, to confirm the occupational and non occupational exposure to low doses. In 24 h urine of the exposed subjects the following indicators were assayed: hippuric acid (exposure to toluol), phenol (exposure to benzol and phenols), trichloroacetic acid [exposure to halogenated aliphatic hydrocarbons (trichloroethylene, trichloroethane)] and vanylmandelic acid (exposure to amyl acetate). The colorimetric-spectrophotometric method described by Tomokuni *et al.*¹⁴ was used to determinate urinary hippuric acid; the urinary trichloroacetic acid concentration was measured following the colorimetric-spectrophotometric method of Grisler *et al.*¹⁵; vanylmandelic acid was measured following the chromatographic and colorimetric-spectrophotometric method described by Wybenga *et al.*¹⁶ and urinary phenol was detected by spectrophotometry following the method described by Yamuguchi *et al.*¹⁷.

We selected a control group of 71 workers in different jobs, occupationally and non-occupationally unexposed to the risk of contact with substances likely to affect the blood or liver (in particular solvents) and homogeneous with the exposed group as regards age, working life and confounding factors (mean age 31.0 ± 6.9 yr, range 21–50); 40 were women (mean age 28.2 ± 5.2 yr, range 21–37) and 31 men (mean age 34.5 ± 7.4 yr, range 23–50). All the control subjects underwent the same laboratory tests as the exposed subjects, but biological monitoring was not performed because the controls inclusion criteria excluded any exposure to solvent.

The statistical analysis included calculation of the mean, standard deviation and 95% confidence interval; differences between the group means were evaluated by Student's t-test. Frequencies in the groups were compared with the chi-squared test with Yates' continuity correction creating contingency tables. The dose-response relationship was evaluated by regression analysis considering hippuric acid as an indicator of the internal dose and the hemathologic indices as indicators of the effect. A significance level of $p < 0.05$ was used. The statistical analysis was performed by using a SOLOTM statistical package (BMDP Statistical Software, Inc).

Results

The results obtained from the questionnaire answers findings did not differentiate between the exposed group and the controls.

RBC were below the lower reference limit value (threshold) in 50.7% of the exposed workers (controls 7% ; $p < 0.001$); Hb was low in 21.5% of exposed workers, (controls 7% ; $p < 0.05$); Hct was low in 44.6% of cases and 8.4% of controls ($p < 0.001$). WBC were the same in both groups and no subjects in the exposed or control group had a PLT below the threshold (Table 1).

Comparing the men separately, 46.6% of the exposed workers had a RBC below the lower limit of normal (controls 6.4% ; $p < 0.005$); Hb was low in 26.6% of cases, compared to 6.4% of controls (not

Table 1. Prevalence of tests below the threshold (lower reference limit value) in the two groups of subjects

	Exposed 65	Controls 71
RBC	33 (50.7%)*	5 (7.0%)
Hb.	14 (21.5%)**	5 (7.0%)
Hct	29 (44.6%)*	6 (8.4%)
PLT	0	0
WBC	10 (15.4%)	7 (9.8%)

* $p < 0.001$, ** $p < 0.05$.

significant); Hct was low in 46.6% of cases (controls 6.4%; $p < 0.005$) and WBC were low in 20% of the exposed workers, as against 16.1% of controls (not significant) (Fig.1); no subjects in the exposed or control group had a PLT below the threshold. Table 2 shows the mean values and 95% confidence interval of the blood indices: among the exposed males, mean RBC counts and Hct were significantly lower than controls ($p < 0.001$), as were PLTs ($p < 0.05$) but the differences in mean WBC and Hb were not significant.

Taking the women separately, 52% of the exposed workers had an RBC below the lower limit of normal (controls 7.5%; $p < 0.001$); Hb was low in 20% of cases, compared to 7.5% of controls (not significant); Hct was low in 44% of cases (controls 10%; $p < 0.001$) and WBCs were low in 14% of the exposed workers, as against 5% of controls (not significant); no subjects in the exposed or control

group had a PLT below the threshold. Table 2 shows the mean values and 95% confidence interval of the blood indices: among exposed females, mean RBC, Hb and Hct were all significantly lower than controls ($p < 0.001$) while WBC and PLT were virtually the same.

In 20% of workers only one variable was below the lower limit of normal (3 males, 20% and 10 females, 20%), compared with 14.1% of controls (6 males, 19.3% and 4 females, 10%); 21.5% had two variables below normal (2 males, 13.3%, and 12 females, 24%), compared with 2.8% of controls (1 male, 3.2%, and 1 female, 2.5%) ($p < 0.005$). Three variables were low in 18.4% of the exposed workers (2 males, 13.3%, and 10 females, 20%) and four were low in 3% (2 males, 13.3%, no females), but in none of the controls.

As far as liver function tests were concerned we found normal findings both in exposed and control

Table 2. RBC, WBC, PLTS, Hct, Hb values in male and female exposed subjects and controls

Group	Males				
	RBC number/mm ³	WBC number/mm ³	PLT number/mm ³	Hb g/dl	Hct %
Mean	453 × 10 ⁴	5893.3	26 × 10 ⁴	14.4	40.8
Exposed SD	653 × 10 ⁴	1627.2	1.7 × 10 ⁴	1.1	3.4
n=15 95%	416.8 × 10 ⁴	4992.4	25.1 × 10 ⁴	13.8	38.9
c. i.	489.1 × 10 ⁴	6794.2	26.9 × 10 ⁴	15.1	42.7
Minimum	290 × 10 ⁴	2200	23 × 10 ⁴	11.8	32
Maximum	520 × 10 ⁴	8200	28 × 10 ⁴	16.0	45
Mean	509.1 × 10 ⁴	6897.4	22 × 10 ⁴	14.4	45.1
Controls SD	31.0 × 10 ⁴	2019.9	5.8 × 10 ⁴	1.1	2.7
n=31 95%	497.8 × 10 ⁴	6156.5	19.9 × 10 ⁴	13.8	44.1
c. i.	520.5 × 10 ⁴	7638.3	24.1 × 10 ⁴	15.1	46.1
Minimum	430 × 10 ⁴	3670	15.3 × 10 ⁴	13.2	39
Maximum	587 × 10 ⁴	11,000	35.2 × 10 ⁴	18.0	53
t-test	<0.001	ns	<0.05	ns	<0.001
Group	Females				
	RBC number/mm ³	WBC number/mm ³	PLT number/mm ³	Hb g/dl	Hct %
Mean	416.0 × 10 ⁴	6050.0	26.1 × 10 ⁴	12.8	36.9
Exposed SD	30.0 × 10 ⁴	1128.4	4.6 × 10 ⁴	0.9	2.3
n=50 95%	407.4 × 10 ⁴	5737.2	24.8 × 10 ⁴	12.5	36.3
c. i.	424.5 × 10 ⁴	6378.7	27.4 × 10 ⁴	13.1	37.6
Minimum	360 × 10 ⁴	4,000	16.5 × 10 ⁴	10.4	32
Maximum	490 × 10 ⁴	8,400	50 × 10 ⁴	14.8	43
Mean	488.3 × 10 ⁴	6407.7	24.6 × 10 ⁴	13.7	41.2
Controls SD	58.3 × 10 ⁴	1392.1	5.1 × 10 ⁴	1.3	3.7
n=40 95%	469.6 × 10 ⁴	5962.5	23 × 10 ⁴	13.3	40.1
c. i.	506.9 × 10 ⁴	6852.9	26.3 × 10 ⁴	14.1	42.4
Minimum	378 × 10 ⁴	2,600	15.8 × 10 ⁴	11	33
Maximum	630 × 10 ⁴	9,020	38 × 10 ⁴	16	48
t-test	<0.001	ns	ns	<0.001	<0.001

Table 3. Urinary biological indicators in the exposed subjects

	Exposed 65 mean $\pm$ SD (range) (reference values)
Phenol mg/l	21.46 $\pm$ 7.94 (3-43) ($<$ 50mg/24 h)
Hippuric acid mg/l	619.97 $\pm$ 273.59 (208-1,480) (200-1,660 mg/24 h)
Trichloroacetic acid mg/l	0.95 $\pm$ 1.07 (0-5.50) ($<$ 5.5 mg/24 h)
Vanylmandelic acid mg/l	5.34 $\pm$ 2.66 (0-11) ($<$ 10mg/24 h)

range = minimum-maximum interval.

subjects. Biological indicators showed no values outside the normal range. The evaluation of the dose-response relationship did not show any significant result (Table 3).

Discussion

As technical methods for preventing exposure in the work place have improved, contact with frankly toxic high concentrations of xenobiotics hardly ever occurs any longer, except for accidents. Workers are mostly exposed to low doses, generally within the limits set for TLVs, especially in the service sectors. There are numerous definitions of a low dose but one of the most widely accepted, by Barnes¹⁸⁾, states virtually that a low dose is defined as such if it is one or two orders of magnitude lower than the dose of a certain substance that produces a clear toxic effect. Exposure to low doses may be toxic not only for the very small numbers of workers who show individual susceptibility, but possibly also for the working population as a whole. There are several grounds for suggesting this: the growing number of occupational and extra-occupational xenobiotics to which workers are exposed may give rise to interactions ("multideterminism"); exposure during each day and working week may be discontinuous, with the possibility of exposure to high doses; the conviction is growing in the scientific community that TLVs and EBI values need to be reconsidered for various reasons (genetic variability, stochastic effects and "multideterminism").

The art restorer's job we investigated offers a good model of these problems. Restorers are in fact usually exposed to low doses on a discontinuous, unpredictable basis, or to occasional, equally unpredictable high doses. In these circumstances it is logical to suspect that some of the substances involved may have pathogenic effects. This study

found that art restorers did in fact have changes in their blood values compared to controls. The values were just below normal for men, and only slightly higher for the women. The difference between the sexes was limited and we cannot say whether it indicates that women are more susceptible. The fact that the abnormal values were only slightly outside the normal range, and deviate very little from the controls, raises several questions: Do these changes have any real clinical significance? Do doses below the current TLVs only affect certain susceptible subjects or should they be considered an expression of risk for the whole exposed population? Can such effects really be detected with non-specific health survey tools such as basic blood tests? In our opinion the clinical significance of these changes for the exposed workers is uncertain and did not induce us to take the dramatic decision of removing the affected art restorers from their work. But our data seem to mean that doses below the TLVs may represent a risk factor not only for individual susceptible subjects but for the whole population. The effects on the blood of the exposure to low doses of solvents may be better detected by a health surveillance protocol, easy to administer in the workplace, than by exceedingly specific and expensive biochemical tests, not feasible in the workplace and not suitable for low dose exposure to different xenobiotics.

The modifications in the blood we observed appear to be related to exposure to the solvents used in restoration, since there was no appreciable difference between exposed workers and controls as regards confounding factors, and since the sample did not include any subjects with pathologies involving changes in the blood.

Our data showed a significant RBC count decrease without any WBC alteration. Toxic blood changes may usually be linked either to a direct effect of xenobiotics on bone marrow or to a direct effect on circulating blood cells: the lack of hemolysis leads us to hypothesize that the toxic effect is related to a bone marrow inhibition and not to a direct hemolytic effect such as described for aniline¹⁹⁾.

Published findings on the effects of solvents on the blood do not allow us to attribute greater importance in determining blood effects to one of the solvents used in art restoring, though a lower RBC count was shown in workers (paint-making industries, painters and mechanics) exposed to low doses of solvent mixtures^{1, 5)}.

The modification of the blood indices we found cannot be ascribed with certainty to one of the xenobiotics to which art restorers were exposed and although the hematologic effects observed are not typ-

ical of early changes due to benzene exposure we have to point out that benzenic impurities—usually present in toluol, xylol and nitro diluents—may exert a toxic effect on blood cells. Benzene is a well established hematotoxic agent that affects both developing blood cells and bone marrow stromal cells. Hematologic findings following occupational exposure include anemia, aplastic anemia, leukopenia, rarely leukocytosis, blood dyscrasias with hemolytic effects, pancytopenia etc.²⁰⁾ Its effects usually occur in three stages ranging from a slight decrease in the number of circulating blood cells to progressive bone marrow aplasia²⁰⁾.

We have to point out that the workers we studied are also exposed to phenols: recently Hsieh *et al.*²⁰⁾ showed in mice exposed to phenol a depression of the RBC count in the absence of any change in the WBC count. Phenol, a primary metabolite of benzene, is more toxic to bone marrow than benzene itself: bone marrow shows high levels of peroxidase for which phenol is a known substrate (probable products are hydroquinone and catechol). Phenol can be activated by co-oxidation with prostaglandins to more reactive substances such as semiquinone and such a mechanism may be responsible for the hematotoxicity of phenol.

As far as the lack of leukopenia is concerned we have to report the study of Ikeda *et al.*²²⁾ which showed that the simultaneous exposure to toluene in rats protects against benzene induced leukopenia. Such an effect has not been definitely proved in humans but it allows us to hypothesize that the multiple exposure to several solvents may lead to not easily understandable metabolic interactions on bone marrow²³⁾.

We found no hepatic modifications, not only because of the low doses but also because of the youth and short working life of the subjects, since it would appear that longer exposure to low doses is necessary to produce liver effects.

During such work as art restoration, involving low doses of solvents, environmental or biological monitoring would appear to be of little use. The difficulty of identifying and quantifying the numerous substances present, the low doses, varying with time, the complexity and high cost of analyzing them all makes it impossible to propose any correct form of environmental or biological monitoring. It does, however, confirm the importance of primary prevention in these cases, focusing on the installation of appropriate equipment and even on non-specific health surveillance.

Biological monitoring findings were normal despite the changes in blood we found confirming that biological monitoring, like environmental

monitoring, is impracticable. It may simply be useful to organize health surveillance for workers exposed to low doses^{24,25)}.

It would appear, however, that before a population exposed to low doses is scheduled for screening, the occupational physician should make a preliminary assessment of the risk, to identify the low-dose exposure that has no effects on the population and/or on individuals and causes no effects in exposed subjects.

The present findings suggest it may be useful for young art restorers to undergo periodic blood screening tests. The protocol employed appears useful for identifying subjects at risk and establishes how often tests need to be made, depending on the extent to which the variables investigated are altered.

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