Effect of remediation measures on exposure and liver detoxification capacity of children

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Abstract

The efficiency of remediation measures in once heavily polluted industrial areas is conventionally assessed by its decreasing pollution burden. To what extent the redevelopment contributes to an improvement in health is important. To have a valid physiological parameter or/and indicator of exposure as well as the methodology to measure both bio- and/or effect-monitoring is decisive.

An epidemiologic cohort study was conducted, whereby kindergarten children from a heavily polluted industrial area and a control one were observed over a prolonged period of time under conditions of redevelopment. The region-specific external exposure was assessed by 27 volatile organic compounds. The effect was assessed with a specially developed stable-isotope-based diagnostic test ([\textsuperscript{15}N]methacetin test) measuring the liver’s detoxification capacity.

The children’s internal load reflected the significant differences in exposure to chlorinated compounds. The ratio of concentrations for the indicator components (chlorinated compounds) was 2.3 for exposed and control children. The pollution-exposed children showed a 6\% lower liver detoxification capacity. A reduction in pollution by about 70\% was reflected in an improved liver detoxification capacity. The difference between polluted and control area was no longer significant.

Prolonged exposure effects to low concentrations of xenobiotics can disturb hepatic functioning. This stable-isotope-based test can be used as a tool to determine health effects of multi-component exposure when the predominant pollutant is taken into account. The presented method appears well suited for bio-effect-monitoring for the purpose of screening and testing the efficaciousness of the remediation activities.

Keywords: children, intervention study, exposure associated liver detoxification capacity, chlorinated hydrocarbons.
1 Introduction

In the years that followed Germany’s reunification, there was great concern in the industrial region of Central Germany (Halle-Bitterfeld-Leipzig-Böhlen), especially as it pertained to the many urgently needed remediation activities and not in the least to reduce the existing adverse health effects as well as prevent further health impairments. Already in the 70’s and 80’s, pollution exposure-associated ill health effects had been observed in this region which could not be attributed to work–related exposure. Studies conducted among children from industrial regions (Thielebeule [12]) or from urban areas (Bredel et al. [1]; Herbarth [4]) are noteworthy in this regard.

In the years after 1990, investigations were started to look for differences in the disease/symptom profile between East and West Germany with the main interest on the development and influences of changes on chronic diseases. Not investigated, yet, but of great societal interest was the question, to what extent changes due to remediation measures undertaken to reduce pollution exposure in the short- or longterm led to changes in the population’s health status (Herbarth et al. [3]).

The efficiency of remediation measures undertaken in once heavily polluted industrial areas is conventionally assessed by its decreasing burden. Important in this respect is to what extent the remediation or redevelopment activities contribute to an improvement in the health status. It is further important to have both a valid physiological parameter (effect-monitoring) and an indicator of exposure (biomonitoring) as well as the methodology to measure the parameters of this bio- and/or effect-monitoring.

Bitterfeld/Wolfen, located in Central Germany, is one of the oldest industrial basins in Germany. Its development began in the mid-19th century when in the region’s large lignite coal deposits were found. The low costs of mining this coal, the clean water of the nearby river Mulde as well as a growing local infrastructure attracted the emerging chemical industry into the area. In the beginning, Bitterfeld’s chemical industry became particularly important for its production of basic chemicals such as chlorine, sodium hydroxide, aluminium and magnesium. Later the spectrum extended and included nearly all areas of organic chemistry and comprised over the years the manufacture of about 5000 different chemicals. At the peak of the industrial production in the 1970s, as many as 32,000 people were employed in the two main production facilities, the Bitterfeld Chemical Plant and the Film Factory Wolfen (Lücke [9]).

The continuous increase in the chemical production, the extension of the product range and the growing synthesis of many ecologically or toxicologically problematic substances such as chlorinated hydrocarbons and pesticides resulted in the creation of a huge waste problem. Enormous quantities of contaminated waste products were usually disposed of directly in nearby abandoned mining pits. Today, about 20 dumping sites of several million tons of lignite ash mixed with chlorinated hydrocarbons are known alone in the Bitterfeld region.

Remediation measures have been ongoing since 1992, including demolition of abandoned industrial buildings and the covering with soil liners of the major chemical waste dumps in 1995/96.
Currently, remediation is focused on the management of contaminated groundwater (Lücke [9]), being a major risk due to the high phreatic rise resulting in local infiltration of residential buildings in Greppin, a town on the outskirts of Bitterfeld.

The production of chlorinated organic chemicals and emissions from the coal-producing (chemical) industry are, besides traffic- and residential (coal-fired) heating-associated emissions, of special importance in this Central German industrial area. That is to say, primary effects of these chlorinated chemicals on the functioning of the liver can be anticipated. Thus, the effects of the large-scaled pollution and improvements of remediation measures, respectively, should be assessed on the basis of exposure-associated changes in the liver function process. Standards (requirements) for such clinical function (detection) tests are high and manifold, as generally healthy individuals, and in this case children, are examined:

- mainly non-invasive tests
- high detection sensitivity of the changes in function
- applicability in epidemiologic field studies

One major difference between biomonitoring and bio-effect-monitoring is, that the latter allows for a prognosis on the effects and on its clinical relevance and does not only provide an assessment based on the toxicological dose-response relationship.

2 Methods

2.1 Intervention study - Study participants

In 1997, all Kindergarten children of one birth year were selected from an industrially heavily polluted village (n=23) and a control one (n=12) with a mean age of 3.3 +/- 0.5 years to be examined during the time of redevelopment. The children were selected to be able to differentiate between exposure related to the parents’ workplace-, the family’s lifestyle- (e.g., smoking) or environmentally-associated factors. This information was elicited by a parent-completed questionnaire survey. During the investigation, each child underwent four intensive medical check-ups and four liver function tests.

2.2 Exposure measurements

2.2.1 External exposure

The region-specific external exposure to chlor-organic compounds were monitored, in total 27 volatile organic compounds (VOC) were measured: alkanes, cycloalkanes, aromatics, organic chlorine compounds and terpenes. From these 27 VOCs, tri- (TRI) and tetrachloroethylene (TETRA) were selected for experimental and statistical analyses because of their known toxicity, their relevance in the liver’s metabolic processes and the existence of their metabolic product, trichloro acetic acid (TCA), which served as indicator of exposure in the liver function test. Exposure was measured outdoors and indoors. Outdoor
exposure was determined within close vicinity of each Kindergarten facility. This was to represent the environment in which the children spent most of their time outdoors except for the time they spent to go back and forth between their residence and the Kindergarten.

Indoor exposure measurements were taken in all children’s residences. As a rule the passive sampling monitors were placed in the living room or in the children's room, i.e., those rooms the parents indicated as places where the child spends most of the time indoors. All measurements were taken twice a year (in the spring and fall) at the same time of the children’s medical checkups.

VOCs were collected with passive sampling monitors (3M monitor, Neuss, Germany). The monitors were installed over a period of 4 weeks in- and outdoors as described above. The adsorbed substances were extracted with carbon disulfide and analyzed using a capillary gas chromatograph system (GC, PerkinElmer, Berlin, Germany). An internal standard (cyclododecane) was used for quantification. The detection limit was 0.1 µg/m³, the precision was more than 90%.

2.2.2 Internal burden
TCA was measured in urine using a modified procedure of the DFG (Deutsche Forschungsgemeinschaft, German Research Foundation) for determination of TCA in blood. Urine was diluted with aqua destillata, given in 22 ml headspace vials and for 2 h in a drying cupboard (90 °C), to split TCA in chloroform and carbon dioxide. Afterwards the quantitative headspace analysis has been applied using the standard additional procedure.

Calibration was effected by the standard addition method to further 10 ml sample (1 ml diluted urine). This double of the sample was spiked with 50 µl aquatic solution of TCA (70 ng/ml) and both samples were thermic splitted and analysed. The peak areas from both samples (ATCAsample, ATCAspiked) were integrated and the TCA burden was calculated according the following equation:

\[
\text{TCAsample} [\text{ng}] = \frac{\text{ATCAsample} \times \text{TCAspiked} [\text{ng}]}{\text{ATCAspiked} - \text{ATCAsample}}
\]

The results were normalized to creatinine to adjust the urinary concentration on variations of the urine volume. Urinary concentration was analyzed using ion-pair-high-performance liquid chromatography with ultraviolet detection (Palmisano [10]).

The detection limit was 0.1 µg/l. The method precision was estimated for TCA using spiked control urine with 89%.

2.3 Clinical part - [15N]methacetin liver function test
To determine whether the children were exposed to organic chlorine compounds, the internal burden of trichloro acetic acid (TCA), metabolite of the indicator components of the organic chlorine compounds was assessed. In order to monitor an effect, the liver detoxification capacity was tested, using the specially adopted diagnostic test ([15N]methacetin test) (Krumbiegel, 1991). This test
measures the elimination rate of an $^{15}$N-labelled drug ($[^{15}$N]methacetin). Knowing the detoxification process of $[^{15}$N]-methacetin and the quantity of the $^{15}$N labelled metabolite excreted in urine over a period of six hours following application, the elimination rate can be calculated. Analyses of the $^{15}$N-labelled metabolite in urine are carried out using an $^{15}/^{14}$N emission spectrometer (Fischer ANalysen Instrumente GmbH, FAN Ltd., Leipzig, Germany).

Test protocol:
- oral application of 3mg $[^{15}$N]methacetin (95% $^{15}$N) per kg of body mass dissolved in a cup of warm herbal tea
- successive collection of all spontaneous urine for 6 hours
- analysis of $^{15}$N in the urine samples
- calculation of the elimination rate of the diagnostic agent relative to $^{15}$N excess amount administered (can also be expressed in terms of elimination half-life)

The test is highly sensitive and reveals early deviations from the expected “normal range” in the liver’s detoxification capacity.

![Figure 1: Principle of the $^{15}$N-labelled methacetin urine test to determine the liver’s detoxification capacity](image)

3 Results and discussion

Contrary to the markers used for assessment of the pollution in general (like sulfur dioxide and dust), which can be relatively easily assessed, exposure to industry-associated chemical mixtures is much more difficult to be evaluated. Considerable differences exist between villages with or without industrial developments and irrespective of whether the industrial company is still producing or has been closed, the contamination of the environment (air, soil, surface water and/or ground water) will remain.

The main remediation activities peaked in 1995/96. Analyses of information gained by the questionnaire allowed us to calculate the children’s external...
exposure time budget. These data suggested that the children normally spent about 90% of their waking hours indoors and only 10% outdoors. Considering this fact, the dose calculated and shown in Figure 2 can be assumed to be the dose the entire population in the polluted (Greppin) and not polluted (Roitzsch) village was exposed to.

Figure 2 depicts the children’s internal load of trichloroacetic acid (TCA) displaying the difference in exposure to chlorinated compounds especially tri- and tetra-chloroethylene. The budget load (TRI + TETRA) was 2.3 times higher in the polluted than the control region. Considering the substances alone, the factors were 1.8 for trichloroethylene and 2.3 for tetrachloroethylene. The majority of TETRA and TRI undergoes oxidation via cytochrome P450s, with a small proportion being conjugated with glutathione (GSH) via glutathione S-transferases (GSTs).

Interindividual variations of these metabolic pathways by polymorph acting P450s and GSTs may explain standard deviations within the groups. We have not included the detection of enzyme polymorphisms as the distribution of polymorph metabolic phenotypes should be similar within the polluted and not polluted Caucasian population.

At the start of the investigation, the children in the polluted area had a 6% lower detoxification capacity than the control group (referred to the expected lower limit of the confidence interval of the normal value to the amount of approximately 71%).

Figure 2: Resulting dose from indoor and outdoor exposure for TRI and TETRA, internal load (metabolite) and difference in the detoxification capacity between an unpolluted (Roitzsch) and contaminated area (Greppin) (mean values or year 1997 and 1998, 95%CI of the standard error of mean).
Figure 3: Time dependent change in pollutant exposure (indicator components TRI and TETRA), internal load (metabolite TCA) and changes in the detoxification capacity of the liver (mean values ± 95% CI of the standard error of mean, p – significance level using Mann-Whitney U-test).

Improvements in the pollution situation, which led to an approximate 53% reduction in the exposure, was reflected in an improved liver detoxification capacity of the non-specific cytochrome P450 system. The time series shows the improvement of the hepatic microsomal biotransformation capacity (Figure 3). The difference above the expected normal value was at that point no longer significant (difference in detoxification capacity 0.6%) between polluted and control area.
4 Conclusions

Children particularly are adversely affected by exposure to environmental pollution which continues for a long period of time. This manifests itself not only in ill health and overt diseases but may cause beforehand premorbid states, i.e., subclinical disturbances of bodily/organ functions. The detection of such disturbances, especially at pollution levels below toxic concentrations, may be an early sign that certain processes may go out-of-control. On the other hand, cessation of an adverse exposure could lead to/ mark the improvement of the bodily/organ function. Since one of the first target organs of exogenic noxes is the hepatic detoxification system, the liver would be an appropriate organ system to test for early adverse effects due to chronic exposure to xenobiotics at low concentrations.

The study reported here did show, that the stable-isotope-based 15N-methacetin liver function test appears to be an instrument able to detect effects in an individual’s detoxification capacity which can be associated with exposure to multiple-component chemical mixtures when the in a particular environment relevant indicator pollutants are monitored (Rehwagen et al. [11]; Krumbiegel et al. [7]). Furthermore, the test can also be used to determine differences in the detoxification capacity in accordance with measured differences in exposure. Since the test method is easily administered and implemented, the test is quite suitable to be used in epidemiologic studies to monitor improvements in the health status of population groups exposed to xenobiotics, such as chlor-organics, following remediation-associated exposure reductions. Thus, this test is recommended for bio-effect-monitoring.

It can be concluded that the method of testing a person’s liver’s detoxification capacity actually allowed the follow-up observation of the efficiency of remediation activities. The improvement in exposure resulted in an improvement of the children’s hepatic detoxification capacity, i.e., in the catching up of the average liver function within the expected normal range. In addition, the results also suggested that the liver function does seem to recuperate relatively easily and over a short period of time with decreasing environmental stress.

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The study was approved by the Ethics Committee of the University of Leipzig. Informed consent was obtained from the parents of the children.

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