# Biomarkers of the effect of VOC exposure: Recent developments

P. Krumbiegel, O. Herbarth, A. Müller, K. Arndt, M. Rehwagen, U. Rolle-Kampczyk
*Department of Human Exposure Research and Epidemiology, UFZ-Centre for Environmental Research Leipzig-Halle, D-04318 Leipzig, Germany.*
*e-mail: krumbiegel@expo.ufz.de*

## Abstract

Biomarkers are convenient tools to characterize 1) specific human exposure to air pollution, 2) responses of the human organism to exposure, and 3) individual variations of both exposure and response.
This paper presents two effect monitoring tests as recent examples to study human responses to exposure and the application of the tests in environmental epidemiology. The common feature of these test methods is the use of the stable isotope tracer technique as a basic working principle.
One of the methods, the *in vivo* [$^{15}$N]methacetin liver function test, has already been approved for use on humans to characterize $SO_2$ load effects on the detoxification capacity of the liver. Its application field has now been extended for use in regions with other air pollution situations such as airborne dust loaded with heavy metals and airborne volatile organic compounds (VOCs). An excellent correlation was seen between dust or $SO_2$ load and liver detoxification capacities.
The other method, a new *in vitro* test system has been developed to characterize the early effects of a VOC as an airborne pollutant on the nitrogen metabolism. Using the cell system *Tetrahymena pyriformis* and the amino acid [$^{15}$N]arginine as a nutrient in the culture medium, the impact of a toluene load can be measured in terms of changes in the ratios and in the $^{15}$N levels of the various metabolites in several pools.

## 1 Introduction

As is known, exposure to environmental pollutants can cause or exacerbate diseases. However, assessing human exposure to ambient toxics and, moreover, assessing the effects of such exposure on public health, are difficult and complex.

The main problems are:
- low and varying concentrations of specific toxics in ambient air;
- a large number of confounding factors hampering the identification of causes of morbidity and mortality (e.g. smoking);
- severe analytical limitations when establishing causal links between exposure and health effects.

Biomonitoring using biomarkers in environmental applications provide a way of definitively identifying causal links.
"A biomarker can be any measurement in or from biological material that defines an exposure or response to that exposure." [1]
Human biomarkers are mostly based on urine, breath or blood samples.

Human biomarkers may be classified in three subtypes [2]:
- **Exposure markers** (of external exposure or of internal load)
  Measurement of a parent compound or its typical metabolite(s)
- **Effect markers**
  Quantifiable response of an enzymatic system or an organ or organism that can be linked to exposure
- **Susceptibility markers**
  Any factor that can vary from individual to individual that could alter the invasion, the residence time, the metabolism of the pollutant, or the biological response.

Our group is engaged in developing new exposure and effect monitoring tests and studying their application to human exposure research and in environmental epidemiology.
In this paper we would like to present some recent approaches to effect monitoring with special stress on the use of the stable isotope tracer technique.

A biomarker of effect is a measurable biochemical, physiological, behavioural or other alteration within an organism which can be recognized as being associated with health impairment or disease. Human biomarkers of effect are ideal for preventive purposes if they are implicated in a toxic mechanism and if they measure change that is still reversible.
Over the years, several effect markers have been proposed for use in liver toxicity, nephrotoxicity and immunotoxicity.

A few years ago, we proposed a stable isotope aided noninvasive liver function test – the [$^{15}$N]methacetin urinary test – to characterize changes in the detoxification capacity of the cytochrome P450-dependent mixed function oxidase system. This test has delivered useful information on the effects of supposed air pollution sources in about 900 applications.
When examining human beings, the manipulation of individual air pollution impact is, of course, impossible. We were able, however, to use the opportunity to measure the small effects of given air pollution mixtures (with $SO_2$ as an indicator component) in certain industrial regions on groups of kindergarten children aged 3–5. Now we shall continue this method of investigating kindergarten groups in regions with other main pollution components.

To test the potential impact of a distinct air pollutant, especially of a volatile organic compound (VOC), cell test systems are very useful. For ethical reason, non-human systems should be substituted for human systems in fundamental research, especially in serial tests designed to assess the response to different doses of various chemical air pollutants. Therefore, the known cell test concept of using the ciliated protozoan *Tetrahymena pyriformis* was performed by our group in two directions:

1) Estimation of changes to physiological parameters
2) Estimation of changes to the protein metabolism of this organism under the influence of toluene as a first example. Once again, the $^{15}$N tracer technique was selected as a tool to obtain insights into the early response of enzymatic processes.

## 2 Investigations and Results

### 2.1 *In vivo* effect biomarker [$^{15}$N]methacetin

At the 2nd Air Pollution Congress in Barcelona, 1994, we published our initial results of [$^{15}$N]methacetin investigations of kindergarten children in an air-polluted industrial region shortly after German reunification in an area which previously lay in East Germany. With $SO_2$ as an indicator component, we showed that the $^{15}$N elimination rate via the urine decreased between 1990 and 1993 in the heavily polluted small town of Moelbis compared to the neighbouring small town of Espenhain that was less polluted. The $^{15}$N elimination rate served as a measure of the hepatosomal detoxification capacity characterized by the activity of the cytochrome P450 dependent oxygenase [3].

Between 1994 and 1997 we again investigated Moelbis and Espenhain risk groups using the same methods. Once more, we started with groups of children initially three years old living in the same kindergartens as the groups studied four years previously.

In correspondence with decreasing air pollution – due to the closure of the most polluting plants – the Moelbis $^{15}N$ values were found to approach the Espenhain values, indicating increasing liver detoxification capacities.

Thereafter we have investigated comparable kindergarten groups in other industrial German regions:

a) Hettstedt, known to be polluted with airborne, heavy-metal-loaded dust, compared to the village of Wippra, only loaded with "home-made" $SO_2$ pollution from individual wood and lignite heating.
   Investigation period: 1995–1998
b) Greppin, near disused plants producing polychlorinated phenols and other volatile organic compounds (VOCs).
   Investigation period: 1997–2000.

As a main result of the Hettstedt study, excellent correlations were seen between the mean detoxification capacities of the children groups on the one side and dust load or $SO_2$ load on the other side (Figs. 1 and 2). Moreover, detoxification capacities predicted on the basis of measured dust + $SO_2$ correlated very good with the measured detoxification values (Fig. 3).

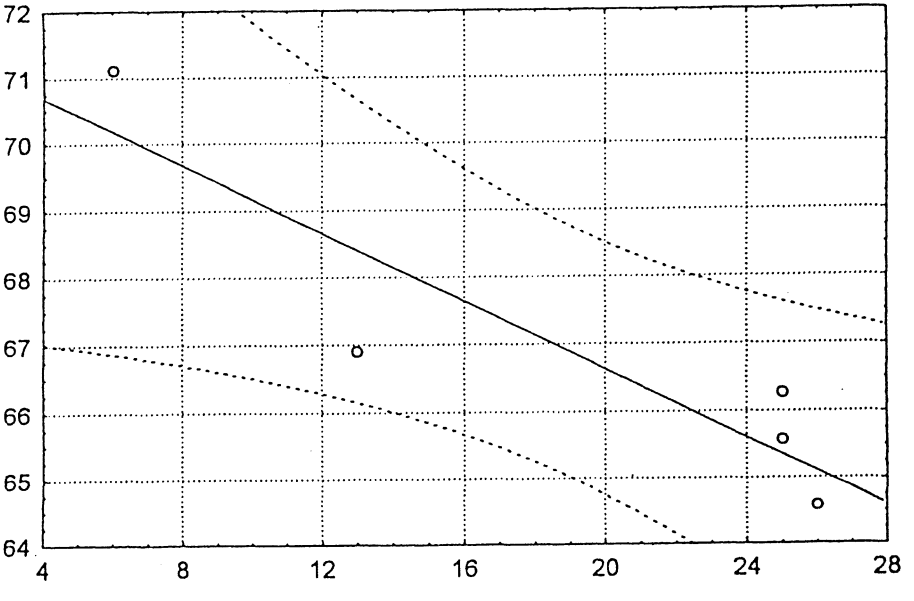


Fig. 1: Relation between detoxification capacities (y-axis [% eliminated $^{15}N$]) and $SO_2$ load (x-axis [$\mu g/m^3$]) in Hettstedt. r = -0.9113; 95% confid..interval

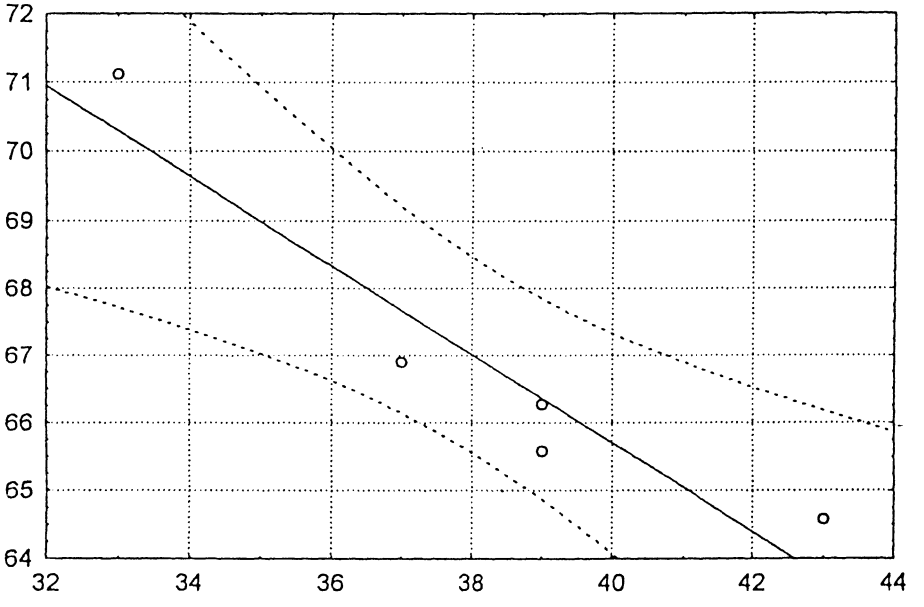


Fig. 2: Relation between detoxification capacities (y-axis [% eliminated $^{15}N$]) and dust load (x-axis [$\mu g/m^3$]) in Hettstedt. r = -0.9470; 95% confid. Interval

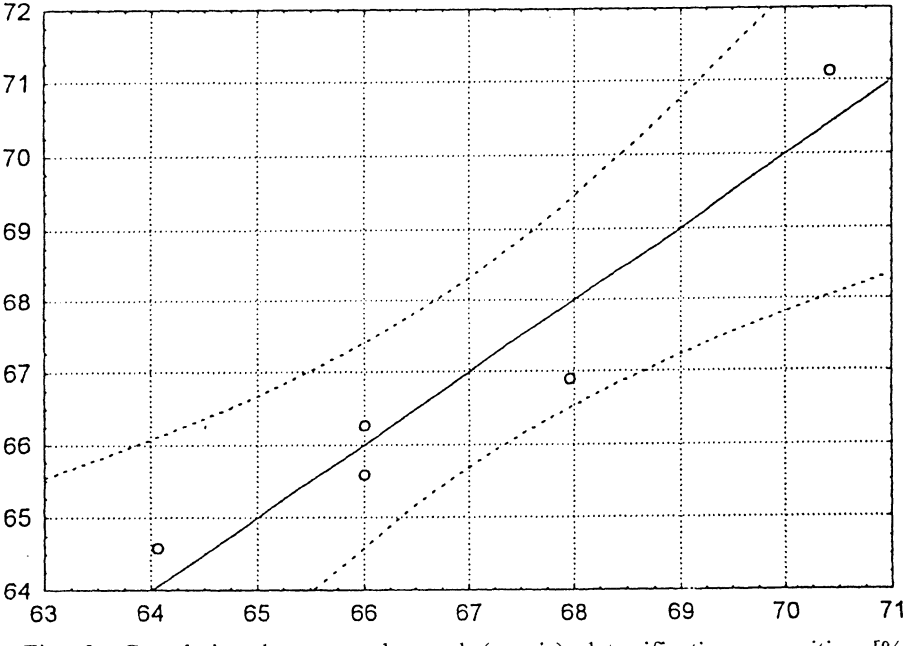


Fig. 3: Correlation between observed (y-axis) detoxification capacities [% eliminated $^{15}N$]) and the values predicted (x-axis) due to dust and $SO_2$ load. In Hettstedt

### 2.2 *In vitro* effect biomarker L-[$^{15}$N]arginine

Prior to the *in vitro* use of stable isotope methods, our group gathered experience with the protozoan *Tetrahymena pyriformis* using this system for *in vitro* effect biomonitoring studies which cannot be undertaken with humans. We estimated changes in physiological parameters such as cell proliferation, cell growth, cell mobility and cell respiration under the influence of certain concentrations of benzene, toluene and xylene. The results of these serial tests revealed that even small amounts of the aromatic hydrocarbons led to a decrease in physiological efficiency [4].

The next aim of *in vitro* effect monitoring should be, however, to measure pollution-caused alterations at an earlier stage than can be seen phenomenologically. We believed that the stable isotope tracer technique would enable insights into elementary steps of protein metabolism. Effects of certain air pollution ought then to be seen in terms of alterations in single steps of the protein metabolism. We expected that the stable isotope $^{15}$N of the biological key element nitrogen should be an excellent marker when added as a metabolic precursor bound to an amino acid such as to L-[$^{15}N_2$]arginine.
L-[$^{15}N_2$]Arginine was added as one of the usual nutrients to the culture medium and its usage and metabolism was observed in the untreated system compared to the same system but under the impact of toluene as a pollutant (Fig. 4).

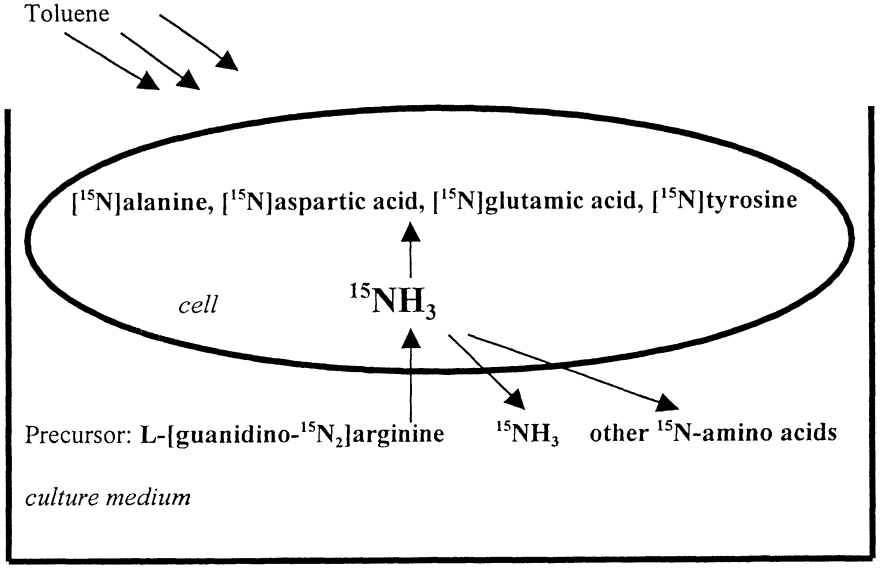


Fig. 4: Tetrahymena pyriformis test system: Principal metabolic ways of L-arginine-borne $^{15}$N

With toluene stress, the digestion of arginine was enhanced. Production and the $^{15}N$ content of ammonia as the end product of the protein metabolism were higher in the toluene-exposed culture by 30% and 40% respectively (Fig. 5). The $^{15}N$-pattern of the free, protein-bound and culture medium amino acids also varied depending on the toluene impact [5].

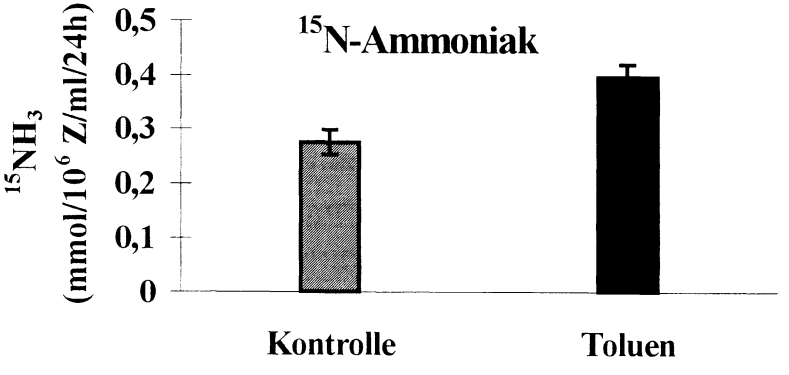


Fig. 5: $^{15}NH_3$ yield without and with toluene exposure [5]

## 3 Conclusion

The *in vivo* [$^{15}$N]methacetin test is now an established effect biomarker, preferably to children. It is a useful extension to human exposure markers.

The *in vitro* [$^{15}$N]arginine method is also intended to be developed to an *in vivo* test in near future.

At present, our methodological work and [$^{15}$N]arginine pilot study offers a method which can produce insights into elementary cell processes which are disturbed under air pollution exposure. In combination with the current non-isotope *Tetrahymena* methods, a comprehensive interpretation of the results - such as disordered anabolism and catabolism, seen, e.g., in terms of reduced cell volume on the one side and higher arginine consumption on the other side - is useful. Combining the results, the suggestion is verified that amongst other things, toxic stress leads to higher energy requirement, resulting in changed metabolism and behaviour.

## 4 References

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