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IMMUNOENZYMATIC SERUM ANALYSIS OF ATRAZINE EXPOSURE AMONG MANUFACTURING WORKERS

Brigitte Scutaru[1], Carmen Cozmei[1], V. Cazuc[1], Doina Popa[1], B. Hock[2]

1. Institute of Public Health Iasi; 2. Technical University München

Abstract. This study assesses the feasibility of an enzyme-linked immunosorbent assay (ELISA) for the determination of atrazine in serum samples collected from twenty workers engaged in technical atrazine dust formulating and bagging processes. An assay was developed with the middle of the test (IC 50) at 0.08±0.02 μg/L, a limit of detection of about 100 ng/L and the coefficients of variation up to 8 %. Serum atrazine levels were interpreted taking into account the airborne concentrations of atrazine in the workplace atmosphere. The immunoenzymatic method itself is convenient, inexpensive and easy to use. It was, therefore, concluded that serum sampling of atrazine exposure among worker populations is a feasible biomonitoring method.
Key words: atrazine, immunoassay, serum samples, occupational exposure

Rezumat. Scopul acestui studiu a fost evaluarea fezabilității unei metode imunoenzimatice pentru determinarea atrazinului în probe de ser prelevate de la douăzeci muncitori implicați în condiționarea şi ambalarea atrazinului tehnic. A fost optimizată o metodă cu punct centrat (IC 50) situat la 0,08±0,02 μg/L, cu o limită de detecție de aproximativ 100 ng/L şi cu coeficienți de variație de până la 8 %. Nivelele de atrazin seric au fost interpretate ținând cont de concentrațiile de atrazin din aerul locurilor de muncă. Această metoda imunoenzimatică este convenabilă, economică şi uşor de aplicat. În concluzie, rezultatele studiului demonstrează faptul că evaluarea expunerii profesionale prin determinarea de atrazin seric reprezintă o metodă fezabilă de biomonitorizare.
Cuvinte cheie: atrazin, metodă imunoenzimatică, probe de ser , expunere ocupațională

INTRODUCTION

Atrazine, [2-chloro-4-(ethylamino)-6-(isopropylamino)-1,3,5-triazine], is one of the most heavily used herbicides over the past 30 years. It is a selective pre- and post-emergent herbicide used to control annual broadleaf and grass weeds, primarily in maize, corn and sorghum crops (20). Atrazine is classified as a hazardous substance according to National Occupational Health and Safety Commission (NOHSC) criteria. Hazardous substances are subject to the workplace controls outlined in the NOHSC Control of Workplace Hazardous Substances (12). The updated EU classification lists atrazine as a class III carcinogen and a class III mutagen (2).

Atrazine is widely produced in America and in some European countries, Romania included.

Since 1987, the product is manufactured in Romania by HERBICIDES *Chimcomplex Company – Borzesti*.

The Romanian atrazine products are available as wettable powders and water dispersible granules (600 g/kg atrazine). During the last four years, the workers have been engaged only in technical atrazine dust formulating and bagging process.

Since 1990, different epidemiological and experimental studies have been carried out in our research group (3,7, 9,16,17,18,19), conducted under the WHO guidelines and included:

- the assessment of the occupational environment in a synthesis and formulating atrazine unit;
- the investigation of the health status of chronically exposed workers;
- experimental studies on two different strains of laboratory animals in order to study thoroughly certain aspects of atrazine noxious potential enhanced in the health status of the occupationally exposed employees;
- the adjustment of a competitive enzyme immunoassay for the determination of atrazine in biological specimens (blood, urine, liver and kidney) taken from acutely exposed rats.

Consecutively to these concerns, this study was conducted to investigate the feasibility of atrazine biomonitoring in serum of exposed worker populations using an enzyme-linked immunosorbent assay (ELISA) method.

MATERIAL AND METHODS

Blood samples were collected at the end of shift, from 20 workers (12 men), aged between 30 and 46 years. The average period of work in atrazine manufacture was 16.45±4.44 years. The serum levels of atrazine were measured using a competitive ELISA technique optimized by our research group for the determination of atrazine in biological specimens taken from acutely exposed rats (19).

The anti-atrazine monoclonal antibody K4E7 and the peroxidase hapten tracer were purchased from the Technical University of München, Department of Botany. Cross-reaction studies with this antibody have been described by Giersch (8). The standard and the other chemicals for the ELISA were obtained from Riedel-de Haen AG, Seelze, Germany. The absorption was read with a microtiter plate reader, Stat Fax 303/Plus, Awareness Technology, Inc., USA. The absorptions were normalized by the transformation to $\%B/B_o$ according to:

$$\%B/B_o = (A - A_{excess})/(A_{blank} - A_{excess}),$$

where:

A= absorption, A_{blank} = absorption at zero dose hapten, A_{excess} = absorption at an excess of hapten.

Each standard and sample was determined three times on two different plates. Data were expressed as the mean ± standard deviation (SD) of six measures. Serum atrazine levels of employees, assigned to various operations, were statistically compared by the "Student's *t* test" and were interpreted taking into account the airborne concentrations of atrazine in the workplace air.

Exposure measurements were performed with a Casella sampling kit loaded with pre-weighted glass-fibre

filters. Airborne atrazine was determined by gas chromatography (15).

RESULTS AND DISCUSSION

Current biological monitoring techniques are often unable to provide accurate estimates of pesticide dose in biological specimens of exposed worker populations. This optimized ELISA method, with the middle of the test (IC 50) at 0.08±0.02 μg/L, achieved a limit of detection of about 100 ng/L. This assay exhibited greatest recognition of atrazine relative to other triazine herbicides.

The mean atrazine concentrations in serum of exposed workers are shown in table 1.

Table 1. Mean concentrations ± S.D. of atrazine in serum samples and coefficients of variation (C.V.)*

Subject No.	Occupation	Atrazine [μg/l]	C.V. [%]
1	bagger	52.93 ± 3.00	5.66
2	formulator	28.36 ± 0.66	2.30
3	bagger	18.96 ± 0.66	3.48
4	bagger	33.76 ± 2.37	7.00
5	bagger	55.06 ± 1.36	2.40
6	team supervisor	14.03 ± 0.82	5.80
7	bagger	35.93 ± 0.49	1.36
8	bagger	33.93 ± 1.84	5.40
9	formulator	7.96 ± 0.46	5.70
10	formulator	13.90 ± 0.80	5.70
11	team supervisor	10.13 ± 0.49	4.80
12	team supervisor	10.80 ± 0.45	4.16
13	bagger	42.83 ± 0.15	0.35
14	formulator	22.63 ± 1.27	5.60
15	team supervisor	6.00 ± 0.70	1.16
16	formulator	29.23 ± 1.43	4.80
17	formulator	15.56 ± 1.20	7.70
18	formulator	9.33 ± 0.46	4.90
19	bagger	63.46 ± 3.75	5.90
20	bagger	11.56 ± 0.89	7.69

* C.V. - variation observed when the same sample was measured on two different plates

The average atrazine concentrations in serum of employees assigned to various operations are presented in figure 1.

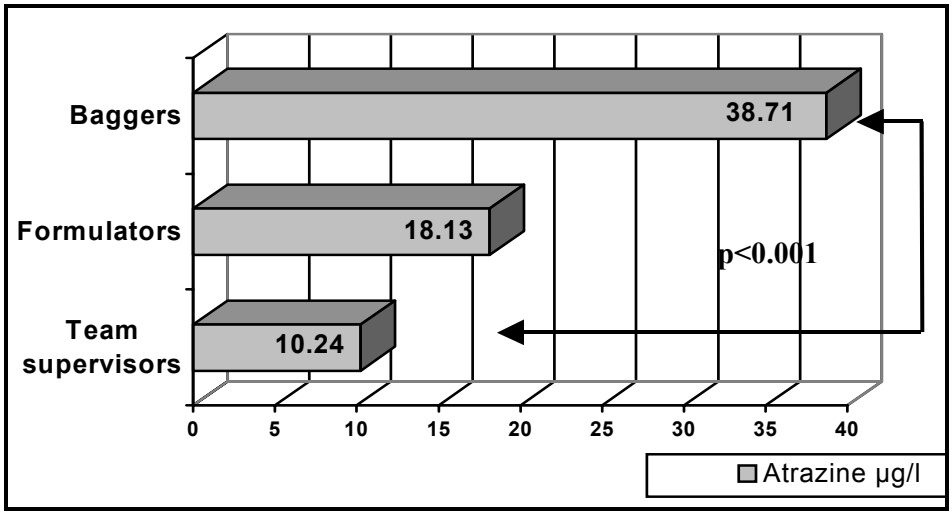

Fig. 1 Average atrazine concentrations in serum by occupation

Serum free atrazine indicates a higher level of exposure in the case of the bagging operators, in comparison with the staff employed in other areas. The difference between baggers and team supervisors is statistically significant (fig. 1).

The atrazine concentrations in breathing area during formulating and bagging varied from 0.96 to 1.73 mg/m^3 (8 h time weighted average). Higher atrazine levels were detected on the final product's exit point from the bunker; therefore, the workers involved in bagging are the most exposed.

We presume that a worker inhales approximately 10 cubic meters of air / shift with a pulmonary ventilation capacity of 1250 L/h (6). According to ACGIH (1), approximately 50% of the airborne atrazine is absorbed upon the inhalant path, depending both on the granulation of the technical herbicide and its solubility in water. In our study, the mean amount of atrazine absorbed during a shift was estimated at 6500 µg/worker. We found a mean atrazine concentration in serum of 25.82±17.24 µg/L, which represents approximately 2.22% of the amount hypothetically absorbed, if we consider that an adult person possesses about 5.6 liters of blood.

These results showed a pattern consistent with exposure to higher amounts during bagging and at the same time, they confirm literature data which specifies that approximately 2% of the absorbed amount of atrazine remains in the form of unmodified parent compound (4,5).

Recently, by using an ELISA test, a mercapturic acid conjugate of atrazine has been identified as its major urinary metabolite in exposed workers. This compound has been found in concentrations at least 10 times higher than those of any other products (10,13,14). A relationship between cumulative dermal and inhalation exposure and total amount of the mercapturic acid conjugate excreted over a 10-day period has been observed (11). Although, the determination of unmodified compounds in biological media may be useful as a qualitative confirmation of the nature of the compound that has generated the exposure.

CONCLUDING REMARKS

- The measurement of biological indicators of exposure is particularly useful for exposed working populations where the conventional techniques of exposure assessment through ambient air monitoring are scarcely applicable (especially when there is a lack of personal dosimetry devices).
- The immunoassay represents a valuable method to detect low levels of pesticides in biological specimens of occupational exposed subjects. This optimized ELISA method, using the monoclonal antibody K4E7, with the limit of quantitation of about 100 ng/L and the CV up to 8%, is a rapid and inexpensive analytical technique, selective to atrazine over the structurally similar triazine herbicides.
- On the basis of these data, we propose this optimized ELISA technique for the determination of serum atrazine, which could be a useful marker of exposure. This method needs further testing on larger samples before it can be used for standard screening.

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