Toxicity Study of Benzene, Toluene and Xylene (BTX) at Exposure on Some Experimental Groups

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Acute toxic potential evaluation of chemicals is necessary to determine the adverse health effects that may occur due to accidental or deliberate exposure to toxic environments. Acute toxicity refers to those adverse effects that may result from polluted environments exposure of experimental animal groups by studying the effects on oral and dermal tracts. The issues investigated are clinical signs of toxicity such as changes in body weight and/or pathological changes in organ and tissue structures which in some cases may lead to death.

Keywords: benzene, toluene, xylene

Toxicity tests regarding industrial polluted environments through controlled exposure on lots of animals have application in studying the harmful effects of pollutants. The information can be used in experimental studies for more precise analyses of the toxicity adverse effects, which can generate severe health problems of the tested subjects.

In conjunction with other tests obtained from experiments on animals one can predict the possible chemicals and combinations thereof, which generate behavioural or health changes, other possible illnesses or malformations on tested subjects.

Experimental results offer useful information that can meet the classification methods for industrial toxicity, standardization of methods for classifying the toxicity, environmental protection and they can provide information on prevention of occupational intoxications.

Statistical techniques are frequently used in data analysis and experiments, and there are also experimental research methods to determine differences between groups which are studied [1].

In this paper, the toxicity studies were carried out by studying the noxiousness of volatile organic compounds, such as benzene, toluene and xylene (*o, m, p* mixtures) and the mixture of the three components (BTX) 1/1/1 (v/v/v).

For toxicity studies (acute, sub acute and chronic), a community of 85 white mice was used, male NMRI strain. The animals were allowed to adjust to the new habitat for 3 days while receiving food and water. These animals were then separated into 4 lots: 3 lots of 20 individuals and one lot of 25 individuals [2, 3].

Experimental part

Materials and methods

The effects of exposure to toxicity were monitored for all these animals. They were exposed to mixtures of benzene, toluene and xylene (BTX) (7) that was inhaled. The animals were exposed to the tested compounds under similar conditions of a possible atmospheric concentration to which people may be exposed as a result of a possible contamination [4-6].

The animals were monitored for 14 days in these aspects: exposure lethality, body weight, motor behavior, aggressiveness and body details such as fur and mucus aspect. These toxicity tests are intended to reveal any physiological and/or pathological changes induced by administration or exposure to toxic substances [8-11].

Toxic exposure of tested animals was done in correlation with time interval, exposure route and toxic dose at which people may be exposed in the event of a possible crisis. The dosages chosen for single exposure toxicity study were as following benzene1g/m^3, toluene1g/m^3, xylene *(o, m, p* mixtures) 1g/m^3 and the mixture of the three components (BTX) 1/1/1 (v/v/v).

At the end of the observation time interval, the CL50% parameters were determined and a statistic analysis was performed on the results (difference in medium exposure) using the Student t* Test and through ANOVA variance analysis. The statistical analysis was performed with Prism 5 for Windows, version 5.01.

CL50= median lethal concentration (concentration that generates death of 50% of the population). The t* test can be used when two samples are taken from the same variable, usually before and after treatment [12].

The relation for obtaining the t_{calc} variable is:

$$t_{calc} = \frac{\overline{x}_1 - \overline{x}_2}{\sigma_{\overline{d}}}$$

where $\sigma_{\overline{d}}$ is the standard error as a difference between the pairs of data and is calculated as following:

$$\sigma_{\overline{d}} = \frac{\hat{\sigma}_d}{\sqrt{n}} \qquad \hat{\sigma}_{\overline{d}} = \sqrt{\frac{\sum (d - \overline{d})^2}{n - 1}}$$

where:

- d is the difference between each pair of measured data;
- $\overline{d}$ is the average between each individual differences (equivalent to $\overline{x}_1 - \overline{x}_2$);
- $\hat{\sigma}_d$ is the best estimated standard deviation of the difference between the pair of measured data.

The only difference in applying this test is the method for determining the degree of freedom:

$$df = n - 1$$

ANOVA is one of the most pertinent statistical processing methods. It is a set of methods for analyzing observed

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data which depend on many concurrent acting factors, in order to determine the most important of them and also to estimate their effects.

The "Prism 5 for Windows" program allows results interpretation through the quintile value *p* which represents a probability and can take values in the interval [0.1]. For example, if $p < 0.05$, it is interpreted that there is a statistical significant difference between the two evaluated populations (help topics, Prism 5 for Windows).

The animals were exposed to the studied compounds in the above mentioned concentration for 5 min in confined space of 0.01 m m^3 in volume. Comparing to day 1, the percentage changes in weight were calculated using the formula [(observation day weight - initial weight) / initial weight * 100].

Experimental part

A preliminary study of animals kept for more than 10 minutes in an enclosed 0.01 m^3 space in normal contaminants-free atmosphere revealed that these animals showed clinical symptoms of psychomotor agitation as sign of hypoxia installation. This is the reason the toxic exposure time was set to 5 minutes – to avoid the real symptoms to be masked by the induced hypoxia.

The tested inhaled dosages were: 1g/m^3 benzene, 1g/m^3 toluene, 1g/m^3 xylene, 1g/m^3 from a mixture of these three compounds in concentration of 1/1/1 m/m/m, as described in figures 1 to 4.

The average change in body weight on each day of 5-minutes exposure to benzene (fig. 1) was recorded in percentage and the average weight on each lot was considered, after 14 days of observation.

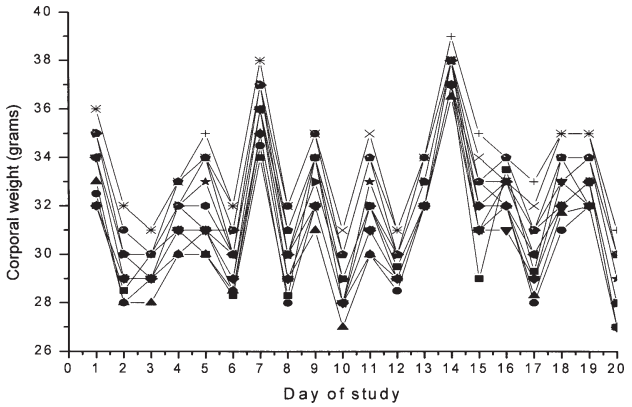

Fig 1. Body weight evolution of individual animals in each day of 5 minutes, 1g/m^3 benzene exposure

After the exposure to the above mentioned conditions, no lethal effect occurred.

Recorded trends in body weight evolution of treated animal were positive (increase) during the experiment.

After experimental exposure to benzene and toluene no changes were noticed in motor or sexual behaviour, aggression or appearance, through all experiment interval. Body weight recorded an upward trend from the 2nd day of observation, gaining statistical significance in 7th day as a proof of disappearance in toxicity clinical signs (fig. 2).

Exposure to xylene did not cause any changes in motor and sexual behaviour, aggression and appearance in the lot during the tracking interval (fig. 3). Body weight recorded an upward trend from the 3rd day of observation, gaining statistical significance in the 11th day as a sign of disappearance of toxicity clinical signs.

The exposure to BTX mixture (fig. 4) also did not cause any changes in motor and sexual behaviour, aggression and appearance of the entire lot during the monitoring time.

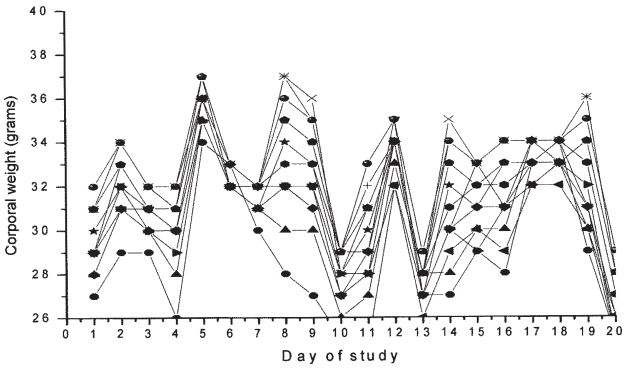

Fig. 2. Body weight evolution of individual animals in each day of 5 . min, 1g/m^3 toluene exposure

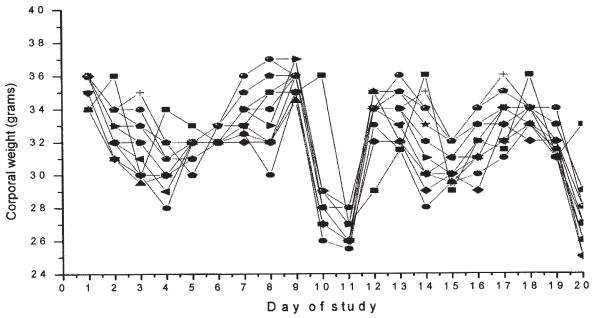

Fig. 3. Body weight evolution of individual animals in each day of 5 min, 1g/m^3 xylene exposure

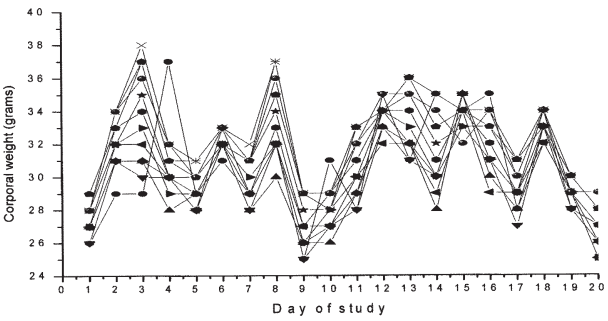

Fig. 4. Body weight evolution of individual animals in each day of 5 minutes, 1g/m^3 mixture exposure

Body weight recorded an upward trend from the 2nd day of observation, gaining statistical significance in the 6th day as a proof of disappearance in toxicity clinical signs.

Conclusions

After this toxicity study of single exposure of animals according to the experimental protocol, the following aspects have been determined:

-after exposure to benzene, toluene and xylene (*o, m, p* mixtures) at specified concentrations (fig.1-4), no lethal effects were recorded;

-after the toxicity study, in the conditions which were presented, animal evolution increased statistically considering body weight. Clinical signs showed complete elimination of toxicity;

-no changes were recorded regarding:

- feeding behaviour;
- motor behaviour;
- aggressiveness;
- mucus and external body (skin, fur) appearances.

The experimental results of this paper can be included in the research domain of new types of reactions at polluting environment through exposure of some lots of subjects.

This paper has application for the identification of new techniques, testing and response methods regarding exposure to contaminated environments.

References

1.L.L. BRUNTON, J.S.S. LAZO, K.L. PARKER- GOODMAN and GILMAN'S, The Parmacological Basis of terapeutics, Eleventh Edition, Mc Graw Hill, 2006;
2.C. D. KLAASEN-CASSARETT and DOULL'S, Toxicology, The basic Science of poisons, 6th edition, Mc Gravw Hill, 2001;
3.A. W. HAYES, Principles and methods of toxicology, Fourth edition Taylor and Francis, 2001;
4.J. B. LEIKIN and F. P. PALOUCEK, Poisoning and toxicology handbook, Lexi Comp, INC Hudson, OHIO, 2002;
5.*** Dirrective 2001/83/CE of European Union Parlament and Council, november 6th, 2001;
6.*** http://www.prc.cnrs-gif.fr/reach/ro/toxicological_data.html;
7.*** Romania Government OM 143/ April 1st, 2002, and No. 400/ may 20th, 2002;
8.*** ANSVSA order no. 84/ August 30th, 2005;
9.*** GAP (Good Animal Practice);
10.*** GLP (Good Laboratory Practice);
11.*** *http://www.sigmaaldrich.com/catalog/DisplayMSDSC;
12.*** EUROPEAN PHARMACOPOEIA Cap. 7.5 Statistical analysis of results of biological assays and tests

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