

## CROTOX '96

Abstracts of the 1st Croatian Congress of Toxicology (with international participation) – CROTOX '96, Zagreb, Croatia, April 17–19, 1996

## FOREWORD OF THE EDITOR

*T*he Croatian Toxicological Society in cooperation with the Institute for Medical Research and Occupational Health, Faculty of Food Technology and Biotechnology within the University of Zagreb, and the Emergency Medical Center, all in Zagreb, organized the 1st Croatian Congress of Toxicology with international participation – CROTOX '96 in Zagreb from April 17–19, 1996.

The participants at the congress addressed a variety of toxicological issues concerning harmful effects of chemicals on human health and environment. This issue of the *Archives of Industrial Hygiene and Toxicology* presents the abstracts in English of the studies conveyed at the congress. In order to reflect the original concept of the congress, the abstracts have been divided in seven sections, as follows: Ecotoxicology, Analytical Toxicology, Biochemical Toxicology, Genotoxicology, Clinical Toxicology, Effects of War on People and Environment, and Free Communications.

## UVODNA RIJEČ UREDNIKA

**H**rvatsko toksikološko društvo, u suradnji s Institutom za medicinska istraživanja i medicinu rada, Prehrambeno-biotehnološkim fakultetom Sveučilišta u Zagrebu i Ustanovom za hitnu medicinsku pomoć u Zagrebu, organiziralo je 1. hrvatski toksikološki kongres s međunarodnim sudjelovanjem – CROTOX '96 u Zagrebu od 17. do 19. travnja 1996. godine.

Na kongresu se raspravljalo o različitim toksikološkim problemima koji se odnose na štetne učinke kemikalija na ljudsko zdravlje i okoliš. U ovom broju *Arhiva za higijenu rada i toksikologiju* tiskani su na engleskom jeziku sažeci priopćenih radova.

Sažeci su razvrstani u sedam skupina: ekotoksikologija, analitička toksikologija, biokemijska toksikologija, genetička toksikologija, klinička toksikologija, učinci rata na ljude i okoliš, te slobodna priopćenja.

Zainteresirani se mogu obratiti izravno autorima za detaljnije podatke o predstavljenom radu.

*RADOVAN PLEŠTINA, glavni urednik*





SECTION 1

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# Ecotoxicology



## THE EFFECT OF ATRAZINE ON HYDRA (*Hydra viridissima Pallas*)

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Our study shows the effect of pesticide atrazine (Radazin T-50, "Radonja" Sisak) on green hydra (*Hydra viridissima Pallas*) with respect to its cytological, histological and morphological aspects. Atrazine is a selective, triazine herbicide that affects the plant photosynthesis. Hydras were treated by atrazine in concentrations of 0.2 g/L, 0.04 g/L for 24 hr. Immediately after the treatment, distinct contractions and damage of tentacles and hypostome were noted in all animals. During the course of the experiment, the budding frequency decreased. Besides morphological, atrazine causes distinct changes in cytological and histological structure of the animals. The exterior mucous layer was damaged along the whole body. The number of interstitial cells in ectoderm was reduced. There were only some epitheliomuscular cells left. Similar to hydras treated with other pesticides, the transdifferentiation of zymogen cells and differentiation of gastrodermal interstitial cells were noted in gastroderm. Cytoskeleton of the cell was also damaged, resulting in dispersion of endosymbiotic algae of the genus *Chlorella* all over the epitheliomuscular cell. On the third day after treatment, hydras recovered completely. They were somewhat smaller than the control samples due to transfer of great amounts of cell material for the regeneration process. Their morphology as well as cytological and histological structure was similar to the control samples, while the green algae *Chlorella* returned to their normal position in the epitheliomuscular cells.

*Key words:* herbicides, Hydra symbiosis, budding, transdifferentiation

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## TOXICITY ASSESSMENT OF DRILLING FLUIDS AND THEIR ADDITIVES BASED ON THE YEAST GROWTH MEASUREMENT USING DIFFERENT METHODS

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**T**he increased drilling activities in offshore waters have stimulated the utilization of marine microorganisms and biological systems for toxicity evaluation of drilling fluids and their components. The study outlines some advantages in using (marine) yeast as test microorganisms in toxicity screening. For example, yeasts are important experimental organisms for modern biological research; they are eucaryotes and provide a convenient model for most of the activities of higher life forms and, as a part of aquatic ecosystem, yeast enables better prediction of potential impact on the environment, especially on selfpurification processes.

The main objective in this work was to quantify the growth of yeast exposed to drilling fluid additives (thinner TX, organic polymer CT, viscosifier D) and drilling fluids (laboratory-prepared SPP and spent fluid KB-2Z). We used different methods such as density gradient plate method, oxygen uptake by immobilized cells, measurement of optical density and biomass dry matter.

The obtained results showed that the choice of the method is limited by physical and chemical characteristics of the tested chemicals and that at least two testing methods should be applied simultaneously to ensure accurate toxicity assessment.

*Key words:* toxicity screening, yeasts, drilling fluids

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## TOXICITY ASSESSMENT OF SOME PESTICIDES BASED ON THE SHORT TERM *in vitro* BIOASSAY

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**I**n search for rapid, simple, reliable and inexpensive toxicity tests for use in the screening of chemical compounds and ecotoxicology monitoring, we tried to apply short term bioassay. Here we report the toxicity assessment of some pesticides. Cidokor, Dikocid and Lontrel were assayed for their toxicity in bacterial and cell cultures by measuring dehydrogenase activity. The assay was based on the reduction of 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyltetrazolium chloride (INT) to INT-formazan. The enzyme activity was measured by spectrophotometer at 490 nm. The EC<sub>50</sub> was derived from the best fit line via regression analysis of the data. The described methods have met all our requirements.

*Key words:* pesticides, toxicity, bioassay, dehydrogenase activity

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## DETECTION OF TOXIC XENOBIOTICS IN THE MUNICIPAL WASTE LANDFILL JAKUŠEVEC

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Samples of dichloromethane extracts from the soil laying under the municipal waste landfill Jakuševac, IP injected to an experimental carp, induced the increase in the benzo(a)pyrene monooxygenase (BaPMO) activity for 1.5 to 7.3 times the natural activity level of its liver. The highest induction of BaPMO activity was obtained through waste extracts. They induced the increase in the BaPMO activity (2.08 pmol benzo(a)pyrenehydroxide (BaPOH)/mg/min) 5.7 to 15.4 times higher than in the control sample (i.e. from 11.87 to 32.04 pmol BaPOH/mg/min), in other words, to the levels of activity induced by IP injection of 13.62 to 36.77 mg 3-methylcholanthrene (3-MC)/kg. This induction of BaPMO activity was caused by the presence of different xenobiotics, and correlated to some extent with the found level of petroleum pollution (predominated by polycyclic aromatic hydrocarbons).

The same dichloromethane extracts from the soil and waste, tested by Ames test using the *Salmonella typhimurium* TA 98 strain with the activation, revealed the mutagenic activity. Moreover, samples of waste collected at several locations contained direct mutagens.

Thus, unlike the majority of tested extracts from environmental samples collected in Croatia (the water extracts from the Sava River and the sediment extracts from the Šibenik area), extracts from soil and waste from the municipal waste landfill Jakuševac revealed the presence of promutagenic and mutagenic substances. These findings were the basis for reaching decisions on the sanitation of the municipal landfill Jakuševac.

*Key words:* carp, benzo(a)pyrene monooxygenase, Ames test, municipal waste landfill

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## ACUTE TOXICITY OF EVAPORATING AROMATIC HYDROCARBONS FOR FRESHWATER INVERTEBRATES

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**T**oxicity of ten evaporating aromatic hydrocarbons was tested on crustaceans *Gammarus fossarum* Koch., *Asellus aquaticus* L., snails *Amphimelania holandri* F r. and *Viviparus viviparus* L. The 96-hour semi-static tests were used. The following chemicals were tested: chlorobenzene, dichlorobenzene, 1,2,4-trichlorobenzene, styrene, alpha-methylstyrene, cumene, xylene, toluene, benzene and ethylbenzene. The concentrations of the chemicals ranged from 0.001 to 0.3 ml/L for the crustaceans and from 0.01 to 10 ml/L for the snails. The LC<sub>50</sub> values were measured by probit method for 24, 48, 72 and 96 hours. The most toxic chemical for crustaceans *G. fossarum* and *A. aquaticus* was 1,2,4-trichlorobenzene (96 h LC<sub>50</sub> was 0.0006 and 0.007 ml/L), while for snails *A. holandri* and *V. viviparus* it was dichlorobenzene (96 h LC<sub>50</sub> was 0.080 and 0.017 ml/L). The least toxic chemicals for all species were xylene and toluene. Multiple correlation showed that the death rate of different test species depended either on increased concentrations of aromatic hydrocarbons or on the length of exposure. In some cases, the time of exposure and the increase of concentration had equal influence on their toxicity. The measured physical-chemical parameters (temperature, pH, total hardness and dissolved oxygen) did not show any correlation with toxicity of tested chemicals.

*Key words:* aromatic hydrocarbons, freshwater invertebrates, acute toxicity

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## TRICHLOROETHENE AND TETRACHLOROETHENE IN THE UNDERGROUND WATER OF THE PUMP PLANT OF SAŠNAK

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The purpose of the nine-year examination of the underground water quality from six Sašnak plant wells was to monitor the content of trichloroethene and tetrachloroethene belonging to toxic halogenated hydrocarbons. The results show that:

- the trichloroethene concentrations have kept falling since 1991, while the tetrachloroethene concentrations have kept rising, which suggests a change in the composition of oil-removing agents used in industry.
- the pollution with trichloroethene has been higher in the southern part of the pump plant (wells B1, B2 and B3) while the pollution with tetrachloroethene has been evenly distributed throughout.

These results have been used to determine optimum amounts of active carbon used for filtering underground water in order to render water potable.

*Key words:* trichloroethene, tetrachloroethene, underground water, Sašnak plant wells

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## DISTRIBUTION OF ORGANOCHLORINE COMPOUNDS IN TREE FOLIAGE

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**F**oliage, particularly needles of coniferous trees, are covered with wax which has the ability to absorb airborne lipophilic pollutants. Foliage is therefore suitable for monitoring levels of pollutants on selected sites. Organochlorine pollutants are of particular interest due to their persistence in the environment and in humans.

The presence of the following organochlorine compounds was investigated: HCH( $\alpha$ -,  $\beta$ -,  $\gamma$ -isomers), DDT (and its metabolites DDD and DDE) and polychlorinated biphenyls (PCB). During 1994 twenty samples of pine-, fir-, spruce-, larch-, thuja- and lime-tree were collected in Ogulin (Gorski kotar) while during 1995 two pine-tree samples were collected on Medvednica and Velebit. Compounds were extracted with hexane/acetone (1:1) mixture and the extracts purified with sulfuric acid and florisil®. Qualitative and quantitative analysis was done by gas chromatography with electron capture detector on two different columns.

All compounds, except *p,p'*-DDT, were found in all analysed samples. The highest concentrations present in all samples were those of PCB (median = 12  $\mu\text{g kg}^{-1}$  dry sample). Other compounds were presented in lower concentrations (medians ranged from 0.3  $\mu\text{g kg}^{-1}$  dry sample for *o,p'*-DDT to 4.8  $\mu\text{g kg}^{-1}$  dry sample for  $\gamma$ -HCH).

*Key words:* organochlorine compounds, foliage, coniferous trees

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## THE RELIABILITY OF IMMUNOCHEMICAL DETECTION OF P-GLYCOPROTEIN IN FISH AS A BIOMARKER OF POLLUTION EXPOSURE

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Recently we found that marine invertebrates *Mytillus galloprovincialis* and *Monodonta turbinata* express a multixenobiotic resistance mechanism mediated by P-glycoprotein (Pgp). Regardless of a few exceptions, the expression of Pgp as detected by Western blot analysis generally correlated with the state of pollution at the collection site. However, Pgp detection in liver of freshwater fish caught in polluted Sava river in May 1993 and May 1995 showed that only 17 out of 32 specimens belonging to 7 species, expressed Pgp of 140 kDa. The concentration of Pgp varied irregularly between the species, as well as between individuals of the same species from 2.5 to 150  $\mu\text{g}$  equivalents of membrane vesicle protein isolated from the cortex of a male bovine adrenal gland / 100  $\mu\text{g}$  of protein. In 14 specimens of 5 species caught at the same site at the River Mrežnica in May 1993 the Pgp level was under the detection limit of 1  $\mu\text{g}$  adrenal gland equivalent / 100  $\mu\text{g}$  of protein. However, of 18 specimens of 6 species caught in May 1995 at the same site, 9 specimens of 4 species expressed the Pgp from 10 to 70  $\mu\text{g}$  equivalents of adrenal gland Pgp / 100  $\mu\text{g}$  of protein. At the same time, not even after 18 days of exposure to the polluted Sava river and to water contaminated over the detection limit with Diesel-2 oil, did the carp *Cyprinus carpio* express Pgp. Since Pgp expression seems not to depend on species or exposure, the detection of P-glycoprotein expression in fish by Western blot analysis alone could not be recommended as a reliable biomarker of exposure to environment pollution.

*Key words:* fish, P-glycoprotein, immunodetection, biomarker

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## RADIATION RISK FROM RADON INHALATION AT THE ISTARSKA TOPLICE HEALTH RESORT

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**R**adon ( $^{222}\text{Rn}$ ), through its particulate daughters, has long been known as an agent causing lung cancer, was present in high concentrations. Elevated radon and its progeny concentrations detected in some spas known as "radioactive spas" may lead to health hazards, especially for the occupationally exposed people.

This paper deals with the determination of radon concentrations and estimations of radiation risk from radon inhalation for occupationally exposed personnel working at the indoor swimming pool and the premises used for medical cure at the Istarske Toplice health resort. The estimated radiation doses ranged from  $1.5 \pm 0.1$  to  $6.3 \pm 0.8$  mSv  $\text{y}^{-1}$ . The analyses of chromosome aberrations in the blood samples of eleven workers were also included in the study.

*Key words:* radon, occupational exposure, spas, chromosome aberration

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## $^{90}\text{Sr}$ IN DRINKING WATER OF THE CITY OF ZAGREB

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**T**he paper deals with the presence of  $^{90}\text{Sr}$  activity in public water supply system for the city of Zagreb and the water from the River Sava. Investigations in both water categories covering the period 1990-95 showed that  $^{90}\text{Sr}$  activities did not exceed the value of  $10 \text{ Bqm}^{-3}$ . The radiation dose to man, received from drinking tap water (2 L/day), was found to be negligible.

*Key words:*  $^{90}\text{Sr}$  activity, contamination, dose

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## RADIATION DOSE INTAKE OF $^{226}\text{Ra}$ IN DRINKING WATER IN CROATIA

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Considering the exceptional radiotoxicity of radium, its long half-life ( $T_{1/2}=1622$  years) and possible detrimental effects on human health, the concentration of this radioisotope in drinking water should be monitored. As an alpha emitter and a homologue of calcium,  $^{226}\text{Ra}$  settles in bones with a high potential for causing biological damage.

After radiochemical separation,  $^{226}\text{Ra}$  in tap and well water samples was determined by alpha spectrometric measurement. We estimated an equivalent dose and used it as the measure of radiotoxicity for the period 1990-95. The annual equivalent dose received by an individual from consumption of well water was found to be one order of magnitude higher than the dose from tap water.

*Key words:* radiotoxicity, radium, equivalent dose

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## TRIBUTYLTIN TOXICITY TESTS USING SEA URCHIN GAMETES AND DEVELOPING EMBRYOS

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**T**ributyltin is a highly toxic synthetic compound used as an additive to the antifouling paints preventing the attachment of larvae and their growth on the submerged surface of ship hulls. The antifouling prevention is based on the continuous leaking of tributyltin in the sea water around the ship hulls acting as a toxic barrier for the fouling organisms. On the other hand, due to its highly toxic and genotoxic properties, tributyltin generates serious harm to the marine environment in general, particularly to the larval and juvenile stages of marine invertebrates from the bottom communities.

The toxicity of tributyltin was evaluated using sea urchin gametes and their early developmental stages as recommended model organisms. Various concentrations of tributyltin were obtained by leakage from painted plates or by addition of pure tributyltin in sea water. The toxicity of tributyltin was estimated by measuring the percentage of unfertilized eggs, the decreased cleavage rate and hatching time and the progressive appearing of various morphological deformations as the most obvious evidence of the toxic effects of tributyltin. However, the most accurate quantitative results were obtained by the measurement of DNA and echinochrome production. Both parameters are not specific indicators of physiological and biochemical activity, but they can be used as a growth rate index. The most vulnerable stage during the sea urchin embryonal development are swimming blastulae and if exposed immediately upon the hatching, the toxic effects can be measured few hours later.

*Key words:* tributyltin, sea urchin, embryonal development

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## HERBICIDE INDUCED CHANGES IN *Hydra viridissima* Pallas

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**W**e investigated the toxic effect of herbicide atrazine on cloned individuals of green hydra (*Hydra viridissima* Pallas) in laboratory conditions. One group of the animals was kept in the light, while the other was kept in the dark, thus having lost a great part of their intracellular symbiont - algae of the genus *Chlorella*. Each group was treated with the solution of 43 mg/L of atrazine (Radazin T-50) for 24 hours. The most prominent morphological and cyto-histological changes were visible during the first two days upon treatment. Hydras treated in light manifested significantly more visible changes than the ones treated in the dark. Almost 90% of the treated hydras had their hypostome retracted and contracted, while the outer mucous layer was damaged. Hydras treated in light stayed contracted longer than the ones treated in the dark. Their tentacles were malformed with round swellings at tips. The budding in the group exposed to light was 46% lower than in the control group, while there was no budding whatsoever in the group treated in the dark. During the first two days upon treatment, we noticed damages of the outer mucous layer and of the mesoglea as well as a decrease in the number of cells in the ectoderm. Similar to most compounds containing heavy metals, irradiation and pesticides, atrazine causes remarkable differentiation and transdifferentiation of the zymogen cells in the gastroderm. The increase in the number of gastrodermal interstitial was also manifest. Hydras which regenerated the damaged body parts had normal morphological and cyto-histological constitution. They were notably smaller, however, especially those kept in the dark since they did not feed during the experiment and absorbed great amounts of their cell material for the regeneration processes.

*Key words:* green hydra, herbicide, transdifferentiation

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## BIOLOGICAL CONSEQUENCES OF THE PRESENCE OF MXR-INHIBITORY SUBSTANCES IN AQUATIC ENVIRONMENT

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Recent evidence indicates that inherent presence of multixenobiotic resistance (MXR) mechanism in aquatic organisms represents a general biological defense mechanism for protection of organisms against both endogenous and environmental toxins. This defense mechanism can be inhibited, or blocked, by chemicals, referred to as MXR-inhibitors, or chemosensitizer. In order to measure the concentration of such MXR-inhibiting substances in aquatic ecosystem, we used the accumulation version of microplate assay with NIH 3T3 mouse fibroblasts transfected with MDR1 gene. The concentration of MXR-inhibitors in XAD-7 water concentrates of the polluted Sava River were found to correspond to 5 ppb, the Korana River to 4 ppb and the unpolluted Dobra River to 2 ppb of verapamil equivalents. The potential of the Korana and the Dobra water concentrates to modulate vanadate-sensitive P-glycoprotein ATPase activity was found to correspond to cyclosporin A MXR-inhibitory features blocking verapamil-stimulated P-glycoprotein ATPase activity, whereas the Sava water concentrate was found to be verapamil-like MXR-inhibitor stimulating P-glycoprotein ATPase activity. Concentrations of 4-5 ppb of verapamil-equivalents of MXR-inhibitors may have ecological significance for aquatic organisms: in *in vivo* experiments, the Sava and the Korana XAD-7 water concentrates (200 ml of water equivalents/20 ml exposure medium) caused the respective 88% and 80% increase in accumulation of rhodamine 123 in the gills of freshwater clam *Dreissena polymorpha*, as compared to a 70% increase caused by known MDR-inhibitor, verapamil (20  $\mu$ M). On the other hand, the concentration of 2 ppb equivalents of MXR-inhibitors of the Dobra water concentrate may be ecologically insignificant for aquatic organisms since it did not cause the increase in accumulation.

*Key words:* MXR-inhibitors, XAD-7 water concentrates, *Dreissena polymorpha*

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## ECOTOXICITY MONITORING - USE OF *Vibrio fischeri*

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The proliferation of chemical substances having the potential to pollute any environmental media (air, land, water), or humans via occupational exposure is considerable. Chemical Abstracts now lists over 12 million substances, and over 100,000 have been notified for industrial use within the European Union. Whilst chemical analytical techniques exist for the measurement of approximately 15% of these chemicals, quantification is even more difficult.

Whilst hazard data, e.g. reliable quality toxicological and ecotoxicological data, have been derived by tests using mammals, fish and other organisms, there are often too few data on the majority of chemicals. Data on many natural products – the toxicity of which in some cases can be more acute than of any industrial chemical metabolites found in wastewater, by-products of combustion either from natural (volcanoes) or manmade (warfare) events are poorly documented for individual compounds or mixtures.

In less developed countries where sophisticated techniques may not be available, or supplies of reagents or even electricity cannot be guaranteed, generic techniques have a great deal to offer. An emission of a chemical will cause adverse effects on organisms providing an opportunity to measure such effects on biological systems. The technique which will be described is the reduction of light output in the marine bacteria *Vibrio fischeri* exposed to a toxicant. Furthermore, its dark variant can be used to obtain mutagenicity data. A chronic test, the results of which compare well with *Ceriodaphnia dubia*, has also been developed. These techniques will be presented.

The development of the principles of Environmental Toxicology Assessment will be reviewed together with the concept of toxic insult as a pragmatic tool in environmental risk assessment, risk management and especially risk reduction.

*Key words:* chemical effects, toxicological and ecotoxicological data, *Vibrio fischeri*, environmental risk assessment

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## EXCRETORY PRODUCTS FROM TROPICAL MARINE ALGAE *Caulerpa taxifolia* INHIBIT A BASIC DEFENCE MECHANISM IN COMPETING ORGANISMS

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Many aquatic organisms possess a basic defense multixenobiotic resistance mechanism (MXR) which is similar to the multidrug resistance mechanism (MDR) found in tumour cell lines. MXR protects aquatic organisms by pumping toxic xenobiotics out of the cell. MDR-reversing substances may annul the protective mechanism of MXR, potentiating thereby the toxicity of xenobiotics. Recently we found that at least part of adverse, repulsive and lethal effects of the rapidly expanding tropical algae introduced into the Mediterranean, *Caulerpa taxifolia*, on the microflora and fauna of the surrounding environment may be explained by the MDR-inhibiting properties of substances that can be found in the invaded seawater or extracted from the algae. The results of our investigations show that dry residues of extract from *C. taxifolia* (CE) significantly enhance the accumulation of calcein AM in NIH 3T3 MDR1 transfected mouse fibroblasts, which is an effect similar to that of verapamil, a model MDR-inhibitor, and opposite to dry residues of extracts from routinely collected samples of marine phytoplankton (PE), a referent plant (EC<sub>50</sub> for CE was 5.94, for verapamil 4.73 and for PE 300 µg/ml). In addition, CE was characterized by its ability to inhibit the activity of verapamil-stimulated vanadate-sensitive ATPase of MDR-protein, which proved that CE possesses cyclosporine A-like MDR-inhibitors. Finally, the marine mussel *Mytilus galloprovincialis* exposed *in vivo* to seawater containing CE, seawater containing excretory products of the algae, and to seawater containing 20 µM verapamil, significantly increased the accumulation of rhodamine 123 in the gills (86%, 106%, and 152% respectively). The result suggests the possible mechanism of ecological effects of *C. taxifolia* and explains its superiority to the competing organisms in conquering the environment.

*Key words:* MXR-inhibitors, *Caulerpa* extract, NIH 3T3 fibroblasts, *Mytilus galloprovincialis*

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## MERCURY, LEAD AND CADMIUM CONCENTRATIONS IN MUSCLES, LIVER AND FOOD OF RAINBOW TROUTS (*S. gairdneri* R.) FROM TWO FISH FARMS IN CROATIA

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**F**ish food contaminated with heavy metals significantly increases heavy metal levels in fish. This fact is particularly important when it concerns fish for human food. The concentration of Hg, Pb and Cd in muscle and liver tissue, as well as in the food of rainbow trout were determined by the AAS method during the period 1978-89. Fish originated from two fish farms in Croatia. Median concentrations of Hg, Pb and Cd in the muscle tissue of the trouts were 0.060 (n=24), 0.14 (n=18) and 0.047 (n=21) mg/g, respectively. Median concentrations of Hg, Pb and Cd in five samples of trout liver were 0.098, 0.80 and 0.142 µg/g respectively. In ten samples of trout food median concentrations of Hg, Pb and Cd were 0.053, 2.50 and 0.587 µg/g, respectively. The statistically significantly higher concentrations of lead ( $P < 0.01$ ) in food than those of Hg and Cd, also led to the higher accumulation in the muscle and liver tissue of the trouts ( $P < 0.05$ ) as compared to the other two metals. Individual concentrations of Hg, Pb and Cd in muscle tissue did not exceed the legally determined maximum acceptable concentration (MAC: Hg-0.5, Pb-1.0, Cd-0.1 µg/g), with the exception of cadmium in a few samples. Individual concentrations of Hg and Pb in the trout food were also below MAC (Pb-10.0, Hg-0.2 µg/g), while in the majority of food samples cadmium concentration was above MAC (Cd-0.5 µg/g).

*Key words:* mercury, lead, cadmium, rainbow trout, trout food

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## CONTAMINATION OF HONEY, WAX AND POLLEN BY GASEOUS FLUORIDE FROM THE ENVIRONMENT

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**T**he earth pollution constantly increases by emission of different pollutants. The emission of gaseous fluoride is great in the areas with heavy industry and dense population.

We studied the influence of gaseous fluoride emission on a honeybee colony. We developed a new method for testing these particular samples, including the preparation for chemical quantification of fluoride ions. This study describes the concentrations of fluoride ions in honey, beeswax and pollen. Our examination determined three pollution levels by gaseous fluoride.

*Key words:* gaseous fluoride, chemical quantification

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SECTION 2

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# Analytical Toxicology



## ANALYTICAL TOXICOLOGY: FROM ENVIRONMENTAL MONITORING TO RESIDUE ANALYSIS

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**A**nalytical toxicology is more than analytical chemistry. Although it does imply much analytical work, it should not stop at a statement about more or less accurate level of a certain chemical in some biological matrix. The toxicologist performing analytical toxicology has to make it clear what conclusions can be drawn from his analysis, and maybe even more important, what conclusions cannot be drawn.

Example 1. Experiments using the barn owl and the buzzard as potential biomonitors for environmental quality have shown that both species have particular characteristics due to, e.g., their food preferences and their specific position in the food web.

Example 2. The use of pesticides in edible plant production and of veterinary medicines in meat production requires extensive residue control. Examples of the results of the past years show that a policy aiming at quality control for which the producer is primarily responsible, has lead to a steadily decreasing level of residues.

Example 3. The use of veterinary medicines and food additives does sometimes result in unexpected side effects. The role of the analytical toxicologist can be illustrated by the carbadox case. It is a food additive aimed at reducing pig diarrhoea, but in doses substantially higher than prescribed, it appears to be lethal due to serious hormonal disturbance.

Generally, the analytical toxicologist has to find the balance between a very reliable analysis (which is often quite laborious and expensive) and the time and money available for performing the analysis; often the two demands are contradictory.

*Key words:* biomonitoring, environmental monitoring, residue analysis, veterinary toxicology

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## ABSORPTION SPECTRUM OF CARBOXYHAEMOGLOBIN DEPENDS ON THE EFFECT OF HEAT ON THE BLOOD

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Changes in the absorption spectrum and the effect on carboxyhaemoglobin concentration as determined by the method of differential spectrophotometry according to Heilmayer and Hüfner were studied in postmortem blood after exposure to temperatures of 55, 60 and 65° C for 15-75 min. The results showed considerable aberrations from the carboxyhaemoglobin concentration in the blood kept at room temperature. Therefore, the routine method of Heilmayer and Hüfner, usually used in toxicology laboratories, is not reliable for determination of carboxyhaemoglobin concentration in the blood of subjects killed in fire.

*Key words:* spectrophotometry, carboxyhaemoglobin, blood, forensic toxicology

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## THE RADIOTOXICITY OF TRITIATED WATER AND TRITIATED HYDROGEN

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**T**ritium in the form of tritiated hydrogen (HT or T<sub>2</sub>) is far less radiotoxic than tritiated water (either HTO or T<sub>2</sub>O). However, when the human organism is exposed to atmosphere containing tritiated hydrogen, the inhaled gas will oxidize into tritiated water. Therefore the limit dose considerations should include the production of tritiated water in the organism, thus decreasing the radiotoxicity ratio between tritiated water and tritiated hydrogen. Accordingly, the limit value of derived air concentration for tritiated hydrogen should be lowered. The estimation of radiotoxicity ratio between tritiated water and tritiated hydrogen, as well as of the derived air concentration for tritiated hydrogen, was based on the HT/HTO GENMOD mathematical model.

*Key words:* radiotoxicology, radiotoxicity, tritiated hydrogen, tritiated water, derived air concentration

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## ANALYTICAL QUALITY CRITERIA AND USE OF ATOMIC ABSORPTION SPECTROMETRY (AAS) IN THE BIOLOGICAL MONITORING OF METALS

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There are obvious reasons for making a thorough investigation of metal concentration in the human body; as nutritional and/or conditioned deficiency of essential elements causes impaired function and damage to the organism so can the body accumulation of essential and toxic elements produce adverse health effects. Many metals and metalloids can affect each others absorption rate and metabolism even at low to moderate exposure levels, particularly in case of a long term exposure. In assessing the concentrations of metals in biological specimens, reliable reference values are needed from healthy, occupationally non-exposed subjects. There are numerous publications which present concentrations of metals in body fluids and tissues, claimed to be typical for the study population. Nevertheless, they can differ for a particular metal and it has become clear that it is difficult to determine which values are typical and reliable. This is the reason why constant efforts have been made for the last few years to establish criteria by which one could compare the published results. The criteria concern methods of sample collection and preparation, analytical methodology, quality assurance and evaluation of data. They are an integral part of the international TRACY project which aims to establish reference values for several metals in human tissues and fluids, also recommended by IUPAC.

Despite the large number of sensitive analytical techniques for trace element determination in biological specimens, AAS is still the preferred analytical technique for up to 30 elements. The adequate use and the scope of AAS in terms of recommended analytical quality criteria will be discussed.

*Key words:* metals, human body, atomic absorption spectrometry, reference values

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## $^{210}\text{Pb}$ IN HUMAN URINE

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Through ingestion of  $^{210}\text{Pb}$ , being the commonest way of contamination, human body absorbs approximately 7% of lead. Through inhalation, the body retains as much as 25-30% of lead. The lead deposits in the bones, while the major amount is excreted with urine.

During the analysis, the daily sample of urine (collected over 24 hours, regardless of the volume) was demineralized by means of  $\text{HNO}_3$ ,  $\text{HClO}_4$  and  $\text{HCl}$ . Stable lead served as the tracer. Hydroxides were removed and phosphates precipitated by adding  $\text{NH}_4\text{OH}$  and  $\text{H}_3\text{PO}_4$ . The remaining impurities were removed by means of fuming  $\text{HNO}_3$ , and the chromates were precipitated by  $\text{Na}_2\text{CrO}_4$ .  $^{210}\text{PbCrO}_4$  and  $^{226}\text{RaCrO}_4$  were separated by the addition of  $\text{HCl}$  and EDTA, followed by reprecipitation of  $^{226}\text{RaCrO}_4$ , whereas the lead remained in solution. The  $^{210}\text{PbCrO}_4$  precipitation was obtained by adding  $\text{NiCl}_2$ . Chemical yield was 75%.  $^{210}\text{Pb}$  is a beta emitter of very low energy (0.2-0.6 MeV), and a period of 30 days is required to establish the radioactive equilibrium between  $^{210}\text{Pb}$  and its decay product  $^{210}\text{Bi}$ . Its beta energy (1.2 MeV) was measured by a low activity beta counter.

The results of radiochemical urine analyses show  $^{210}\text{Pb}$  activity of 38 mBq/24 h, measured in a control group of subjects who have never been exposed to lead contamination. The contribution of the described procedure is in the reduction of time required for the analysis by substituting the standard wet-washing procedure with phosphate precipitation method.

*Key words:* radiotoxicology, lead, urine

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## THE IMPORTANCE OF MICROBIOLOGICAL EXAMINATION OF AIR-CONDITION SYSTEMS IN SICK-BUILDING SYNDROME

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Sick-building syndrome (SBS) is a widespread, relatively unknown and generally underestimated phenomenon in occupational and environmental health. Due to a number of SBS cases admitted and examined at the Basel Toxicological Institute ever since 1991, we were able to develop such analytical examination methods as to accurately detect symptoms reflecting health and behaviour disturbances of patients exposed predominantly in air-conditioned buildings. We established the following methodological approach, by steps:

- inquiry of the affected persons as to the number and frequency of physical complaints;
- checking criteria for good health condition;
- examination/evaluation of all building facilities (gas, water and electricity systems);
- microbiological examination of indoor air ventilation systems; and
- recommending restructuring and servicing.

The results of microbiological examination showed that an important factor in SBS is microbial contamination. The Basel Toxicological Institute has developed a cost-effective testing strategy which gives reliable results. It regards microbiological testing of indoor air ventilation systems (such as filters, distribution channels, heat rewinding systems and humidifiers) in order to detect possible microbial contamination and consists of: a) semiquantitative determination of germ counts (microbial content of mold/bacteria), b) qualitative differentiation of germ species (e.g. *Aspergillus*), c) microbiological examination of the water in humidifiers (e.g. legionnaire's disease). This method helps defining certain risks like legionnaire's disease and mould fungus allergies and minimizing them proposing adequate servicing.

*Key words:* SBS, air condition, microbiology, legionella, mould allergy, *Aspergillus*

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## HAIR ANALYSIS AS A DIAGNOSTIC IMPROVEMENT IN DETECTION OF HEAVY METAL POISONING

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**W**e compared and evaluated different analytical methods for detection of heavy metals. Heavy metals (HM) can lead to acute or chronic poisoning. The toxicologists are confronted daily with the question of how to recognize HM intoxication. Successful therapy requires a fast diagnostic method that can detect HM. Acute HM intoxication requires blood test (BT), urine test (UT) and excrement test (ET), especially in cases with previous HM poisoning. Chronic HM poisoning, however, often renders negative results for BT and ET. Consequently, an accurate diagnosis of HM poisoning requires daily repeated BTs for a longer time period. UT cannot be the main method for testing acute HM poisoning, since many HM (e.g. thallium) are hardly excreted through the urinary tract. The chewing gum test (CGT) is used to recognize an increased release of Hg, Zn, Cu and Ag (amalgam) into the saliva. CGT measures only the actual exposure and not the intake. In acute poisoning, the dose plays a decisive role whereas in chronic poisoning other factors might be crucial such as time of exposure, risk factors like anomalies of metabolism, concomitant disease, preexisting organ damage, drugs, and environmental factors. Chronic poisoning is usually preceded by deposition of HM into deep compartments. Our study presents a strategy that allows access to deep compartments *in vivo* in order to identify HM and its concentrations. The analysis of HM in hair or nails by atomic spectrometry (HMA) is a noteworthy alternative to classical methods and invasive biopsy.

*Key words:* heavy metals, acute poisoning, chronic poisoning, hair analysis HMA, humans, *in vivo*

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## EVALUATION OF ANALYTICAL METHOD FOR QUANTIFICATION OF NIFEDIPINE IN PLASMA

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*I*n order to determine nifedipine in plasma, we developed a method using high performance liquid chromatography (HPLC). Nifedipine was extracted from alkalinized plasma with a mixture of dichloromethane and n-pentane (1:1 v/v). The sample was passed through  $\mu$ Bondpack TM C18 column in the normal phase mode by mobile phase of water and methanol (45:55 v/v). Quantification was done by external standard method using UV detection at 235 nm. Standard curve linearity was determined by calculating the regression line coefficient using the least squares method on the basis of 10 calibration curves obtained on different days. The ratio between the measured and prepared drug concentrations was  $0.0183 \pm 1.004$  °C with regression coefficient of 0.999. LOD and LOQ were 0.427 and 0.858 ng/ml respectively. The comparison of the curves of different matrixes with the original calibration curve shows parallelism and superimposition within the established limits of analytical variation. The extraction yield throughout the concentration range was about 75%. The established simple extraction procedure and the speed of chromatography make this method suitable for routine determination of nifedipine.

*Key words:* nifedipine, HPLC

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## MEASUREMENT OF THE DISTRIBUTION OF *m*-XYLENE IN RAT TISSUE

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**B**oth direct analysis of tissue, as well as PBPK (physiologically based pharmacokinetic) modelling, have been used to determine the fate of air pollutants in the biological system, and to provide information for health risk assessment. Although various analytical methods have been used to determine the occurrence of aromatic hydrocarbons in tissue, as well as to estimate tissue: air partition (distribution) coefficients ( $K_D$ ) for use in PBPK modelling, most methods have been cumbersome and labor intensive.

An automated head space-gas chromatography (HS-GC) method was developed and evaluated for reliability in the measurement of *m*-xylene in rat tissue. The analytical precision was better than 12% for blood, brain, muscle, fat, liver, and kidney over the concentration range of 0.1 to 100  $\mu\text{g}$  of xylene per g of tissue. When aromatic hydrocarbon co-pollutants were present in the vial used for head space analysis, the analytical results were not affected except for blood (50  $\mu\text{g/g}$ ) and fat (100  $\mu\text{g/g}$ ) at the highest concentration. For rats sacrificed immediately after exposure to 1100 ppm airborne *m*-xylene in a glass inhalation chamber, the relative concentrations of the *m*-xylene in tissue analyzed by the automated HS-GC method were brain  $\leq$  blood  $\leq$  kidney  $<$  liver  $\ll$  fat.  $K_D$  values calculated from the experimental data were at least within the same order of magnitude as previously published values. The experimental results are useful for estimating the internal chemical doses which would have direct relevance to the biological effects caused by inhalation exposure in animals and humans.

*Key words:* analysis, distribution, tissue, *m*-xylene

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## QUALITY ASSURANCE OF TRACE ELEMENT ANALYSES IN HUMAN BIOLOGICAL SPECIMENS

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**I**n order to establish more reliable reference values (Re: international project TRACY) and ensure analytical quality of data, trace elements in human biological specimens should be determined in accordance with well defined criteria. Quality assurance should cover the whole analytical method, including sampling, storage and sample preparation. The selection of the proper indicator in the proper biological specimen at the proper time and in the proper population has the key role. A daily control of analytical accuracy would be preferable. Ideally, the reference materials for analytical quality control should have both a matrix and an analyte concentration similar to the samples to be analyzed, i.e. two or more reference samples with different levels of the analyte should be used. In addition to the use of internal quality control to monitor technical and methodological errors in an analysis, external quality assessment or interlaboratory analyses of unknown specimens provide a check of analytical compatibility and comparability. Precise and accurate measurements of trace elements are necessary for studies on combined exposure to metals in man, interactions between various essential and/or toxic metals and metalloids, and corresponding health effects. Therefore, our laboratory is included in several external quality assessment schemes and applies various commercially available reference materials to ensure accuracy of trace element determination in human biological specimens by means of atomic absorption spectrometry.

*Key words:* internal quality control, external quality assessment, trace elements

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## PHENOL IN URINE AS AN INDICATOR OF BENZENE EXPOSURE

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**D**uring the period 1987-1990, over 300 urine sample pairs collected from benzene production workers were investigated. The samples were collected before and after work and were analysed by means of gas chromatography for the presence of phenol as an indicator of benzene exposure during work.

In 75% of the analyzed samples, the phenol in urine was up to 10 mg/L, in 18% between 10 and 20 mg/L and in 7% between 20 and 142 mg/L.

Some of the special cases were discussed.

*Key words:* benzene exposure, phenol, urine, gas chromatography

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## IDENTIFICATION OF POLYCYCLIC AROMATIC HYDROCARBONS BY GAS CHROMATOGRAPHY RETENTION INDICES CALCULATED BY CUBIC SPLINE METHOD

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**I**n a situation where sophisticated analytical equipment combining chromatographic and spectroscopic techniques like gas chromatography-mass spectrometry and gas chromatography-Fourier transform infrared spectroscopy are not available, identification is usually performed by the use of retention indices. The oldest one is Kovacs index, primarily intended for isothermal gas chromatography. Polycyclic aromatic hydrocarbons (PAHs) extracted from particles in the atmosphere comprise a number of substances that differ significantly in molecular weight due to different number of condensed rings and different substituents. The efficient separation may be achieved only by temperature programmed gas chromatography. In such conditions, a special system of retention indices for identifying PAHs was developed based on the comparison of retention time of unknown substance with retention time of naphthalene, phenanthrene, chrysene and picene. The lack of picene standard may disable identification of PAHs containing more than five condensed rings that exert the most prominent genotoxic activity. This was the reason to introduce cubic spline interpolation for calculation of retention indices instead of linear interpolation. The "calibration" was performed using the available standards of PAHs. The method of cubic spline was checked by calculating retention indices of PAHs the previous calculation of which was wrong. Subsequently, it was used for identification of PAHs extracted from particles collected in a highly polluted industrial area. This study presents the list of identified PAHs and compares Kovacs indices, PAH indices calculated by linear interpolation and cubic spline, and indices cited in literature.

*Key words:* polycyclic aromatic hydrocarbons, identification, retention indices

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## A NORMAL-PHASE HPLC METHOD FOR DETERMINATION OF DIMENHYDRINATE

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We applied a high performance liquid chromatography (HPLC) method to determine dimenhydrinate (diphenhydramine salt of 8-chloroteophylline) in plasma. Dimenhydrinate was extracted from acidified plasma (pH 2.7) with a mixture of chloroform and 2-propanol (7:3 v/v). A CN-LiChrosorb column in the normal phase mode was utilized with a mobile phase of cyclohexane and 2-propanol (98:2 v/v). Quantification was done by external standard method using UV detection at 215 nm. Standard curve linearity was determined by calculating the regression line coefficient using the least squares method on the basis of 10 calibration curves obtained on different days. The precision of the method was expressed by variation coefficients (1.98 mg/L - 6.55%; 3.96 mg/L - 3.57%; 7.93 mg/L - 2.32%). The assay procedure was applied to determine the bioequivalence of two prepared dimenhydrinates.

*Key words:* dimenhydrinate, bioequivalence, HPLC

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## DISTRIBUTION OF PCB CONGENERS IN HUMAN MILK

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Structure-dependent differences in the toxicity of PCB (polychlorinated biphenyls) congeners and similarities with PCDD (polychlorinated dibenzo-p-dioxins) and PCDF (polychlorinated dibenzofurans) congeners initiated the isomer specific analyses of PCB in biological materials as a basis for risk assessment in the cumulative exposure to those organochlorine contaminants. No data on PCB congeners in human samples have been available in our country so far.

The following PCB congeners (marked according to IUPAC) were measured in human milk samples: PCB-28, PCB-52, PCB-101, PCB-138, PCB-153 and PCB-180. During 1995, ten human milk samples were collected from nursing mothers living in Zagreb. Mothers were not exposed to PCB more than the general population. Compounds were extracted with a mixture of chloroform/methanol (1:1), the extracts were purified with concentrated sulfuric acid, and the quantitative and qualitative analysis was performed by gas chromatography on two capillary columns.

Depending on the compound, the method recoveries were between 49-98% and detection limits between 0.02-0.09  $\mu\text{g kg}^{-1}$  milk. PCB-138, PCB-153 and PCB-180 were found in all analysed samples. PCB-101 was found in eight samples, while PCB-28 and PCB-52 in five samples. The congeners were found in the following concentration ranges ( $\mu\text{g kg}^{-1}$  milk fat): 3.3-10 for PCB-28, 17-64 for PCB-52, 13-32 for PCB-101, 27-145 for PCB-138, 37-141 for PCB-153 and 0.60-9.5 for PCB-180.

*Key words:* PCB, human milk, distribution

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SECTION 3

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# Biochemical Toxicology





## OCHRATOXIN A REDUCES THE HSP70 LEVEL IN RAT LIVER

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Ochratoxin A (OA), a mycotoxin produced by fungi of the genus *Penicillium* and *Aspergillus*, is a potent immunosuppressive, carcinogenic, teratogenic, and genotoxic agent. Exposure to OA causes morphological and functional changes in kidney and liver of experimental animals.

We have examined the effects of OA on the expression of heat-shock protein 70 (Hsp70), a member of a highly conserved group of intracellular proteins the synthesis of which increases in response to a variety of stress stimuli. Fisher rats were treated by subchronic dose of OA (120 µg/kg b.w./day) for 10 days (group A, n=8), and for 35 days (group B, n=8). The control group (group C) was represented by 8 untreated rats.

Hsp70 has been detected in liver homogenates by SDS-PAGE followed by immunoblotting using specific mAb against Hsp70 family.

Our results showed that the amounts of Hsp70 present in livers of animals from group A and group C were similar. However, the amounts of Hsp70 in livers obtained from animals treated by OA for 35 days (group B) were lower.

The reduced expression of Hsp70 caused by OA could be explained by a generally lower rate of protein synthesis in the presence of OA or by other unknown mechanism which participates in the control of expression of Hsp70. This fact raises very interesting questions, such as, how does the decreased amount of Hsp70 contribute to the development of morphological and functional changes in liver of animals exposed to OA?

*Key words:* ochratoxin A, liver, Hsp70

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## RAT UTERINE CELLS: *in vitro* RESPONSE TO S-TRIAZINE

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Cellular models can be of great advantage in the exploration of the mechanism, metabolic profile and potential toxic effects of xenobiotics. They require small amount of substance for testing, are relatively simple and fast, and render valuable scientific information at a reasonable cost. Primary cell cultures retain the characteristic expression of cellular functions, while the cell lines express their original phenotype only in part. Our previous results have shown that the treatment of a rat with atrazine *in vivo* induces prolonged oestrous cycle, characterized by extended vaginal diestrous. The aim of this study is to explore metabolic effects, cytotoxic effects, morphological and ultrastructural changes present in the rat uterus during oestrous, diestrous or proestrous phase after atrazine treatment. Morphological and ultrastructural differences are followed by histological and histochemical examinations of uterus. The uterine cells were prepared from 90 days old rats and  $1 \times 10^5$  cells/walls pipetted on Falcon pre-treated 24-wall plates. Triplicated samples with the equal number of cells for seeding were stored for protein determination. Twenty hours after the seeding (day 1), the medium was removed and replaced with a fresh one. The medium was changed again on day 4, and the cultures were completed on day 7. Atrazine solution in ethanol, ranging from 0.01 to 1  $\mu\text{mol}$ , was added to the cell medium. The presence of atrazine influenced vaginal diestrous through hypothalamus-pituitary and ovary. It seems that atrazine does not have direct influence on the physiology of uterine cells.

*Key words:* cellular models, atrazine, uterine cells

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## CHANGES IN SERUM PROGESTERONE CONCENTRATION ASSOCIATED WITH CYSTIC OVARIAN FOLLICLE DEGENERATION IN PIGS AFTER s-TRIAZINE HERBICIDE (ATRAZINE) TREATMENT

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**F**unctional impairments of the ovary are the major cause of infertility in pigs during intensive breeding. Anoestrus is the common consequence of these disturbances that may be induced by substances from food, such as phycotoxins, mycotoxins, insecticides or herbicides. The aim of this study was to determine whether subacute exposure of sows to low doses of atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine), an s-triazine herbicide, could influence the endocrine regulation of their oestrus.

Sows, 6-7 months old, wt. 80-100 kg, were given 100 and 150 mg of atrazine per kg of diet for 19 days. During the first five post-treatment days anticipating the oestrus period, blood samples were collected to determine serum progesterone (P) concentration by Delfia time resolved fluoroimmunoassay. Paraffined sections of ovaries were stained for histologic examination.

The atrazine-treated animals failed to show the first signs of the anticipated oestrus. Histologic examination revealed the development of many large ovarian follicular cysts and the persistence of corpora lutea. As compared to controls, atrazine-treated sows demonstrated a significant increase in serum P concentration ( $P < 0.05$  and  $P < 0.001$ ) 48 and 24 h before the next anticipated oestrus.

The results showed that atrazine affects the ovarian function in pigs, causing anoestrus. Furthermore, a higher serum P concentration may provide useful data in the diagnosis of follicular cystic ovary disease.

*Key words:* herbicides, atrazine, pigs, oestrous, anoestrous, progesterone, follicular cystic ovary disease

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## MECHANISM OF PHOSPHATURIA IN CADMIUM- TREATED RATS

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The kidney plays a central role in the homeostasis of inorganic phosphate ( $P_i$ ).  $P_i$  filtered in glomeruli is reabsorbed up to 80% along the proximal tubule (PT) by the apically located sodium-dependent  $P_i$  transporter ( $NaP_i$ ). Cadmium (Cd) is a nephrotoxic agent which impairs the reabsorptive and secretory functions of PT cells, as manifested by phosphaturia, proteinuria, aminoaciduria, glucosuria and polyuria in man and experimental animals. The cellular mechanisms of Cd nephrotoxicity are poorly understood. In this study we investigated the effect of Cd on  $P_i$  transport in the PT at the cellular level. Wistar strain rats were treated with  $CdCl_2$  (2 mg Cd/kg b.w.s.c.) for 14 days. Compared to saline-treated control animals, the Cd-treated rats excreted significantly more urine, urinary protein and  $P_i$ , thus indicating a drastic impairment of  $P_i$  reabsorption in the PT. As studied by the rapid filtration technique in isolated renal cortical brush-border membrane vesicles (BBMV), the Na-gradient driven intravesicular uptake of radiolabeled phosphate and glucose was markedly decreased in Cd-treated animals. However, the uptake of radiolabeled sulfate in BBMV from Cd-treated rats was not different from controls. Western blotting (WB) of BBMV proteins and indirect immunocytochemistry in frozen sections of fixed kidneys, using an antibody against the  $NaP_i$ -2, an isoform of the  $NaP_i$  cotransporter present in the rat kidney brush-border membrane (BBM), showed a significantly reduced expression of this protein in Cd-treated rats. Contrary to the above, the WB labeling of water channel aquaporin 1 in BBMV from control and Cd-treated rats was similar. The northern blot analysis demonstrated that the expression of 2.7 kb  $NaP_i$ -2-related mRNA was strongly reduced in Cd-affected kidneys. Our data indicate that (a) Cd reduces reabsorption of  $P_i$  in the PT by affecting the transcription-dependent expression of the functional  $NaP_i$  cotransporter in the apical cell membrane, and (b) the effects of Cd on BBM transporters are selective.

*Key words:* cadmium, phosphate transport, phosphaturia, kidney, rat

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## KINETICS OF ACETYLCHOLINESTERASE INHIBITION BY DICHLORVOS AND TERBUFOS IN RAT BRAIN

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The exposure to organophosphorus pesticides lead to cholinesterase inhibition and to accumulation of acetylcholine in the central nervous system. We studied the effect of dichlorvos (2,2-dichlorovinyl dimethyl phosphate) and terbufos (S-tert-butylthio-methyl-O,O-diethyl phosphorodithioate) on the activity of acetylcholinesterase (AChE) in male rat brain cytosol. The  $K_M$  and  $v_{max}$  values for AChE were determined ( $K_M$  4.21-7.46  $\times 10^{-5}$  M;  $v_{max}$  0.0221-0.0356  $\times 10^{-6}$  mol min<sup>-1</sup> mg<sup>-1</sup>). The activity of AChE was measured by the method of Ellman et al. We studied the influence of the various concentrations of dichlorvos (0.0025-0.1000 mM) and terbufos (0.01-0.25 mM) *in vitro*. The dichlorvos  $v_{max}$  value was  $0,0259 \pm 0,003 \times 10^{-6}$  mol min<sup>-1</sup> mg<sup>-1</sup> and  $K_M$  0.354-0.416  $\times 10^{-3}$  M, while terbufos was  $v_{max}$   $0.0208 \pm 0.0014 \times 10^{-6}$  mol min<sup>-1</sup> mg<sup>-1</sup> and  $K_M$  0.0457-0.1757  $\times 10^{-3}$  M. The kinetic *in vitro* study of the AChE inhibition in the rat brain by dichlorvos and terbufos showed their competitive nature.  $K_i$  values for dichlorvos were  $0.0059 \times 10^{-3}$  M and for terbufos  $0.0114 \times 10^{-3}$  M. Hill's coefficients ( $n_H$ ) for dichlorvos were 1.01 and for terbufos 0.92, suggesting that one molecule of the compound reacted with one molecule of AChE. The values of  $I_{50}$  for dichlorvos were 3.14 mM and for terbufos 220.33 mM.

*Key words:* acetylcholinesterase, dichlorvos, terbufos, rat brain

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## PESTICIDE TOXICITY AND REPRODUCTIVE BIOCHEMICAL MECHANISMS

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**E**xogenous compounds, like pesticides, cause serious failures directly involved in reproductive processes. We tested the toxicity of the commonly used selective liposoluble pesticides (s-triazine herbicide, atrazine and organochlorine insecticide, lindane) on the biochemical mechanisms responsible for normal function of gonadotropic feedback mechanism. Androgen  $5\alpha$ -reduced metabolites act as the intracellular mediators for several reproductive biochemical mechanisms. *In vitro* experiments showed that atrazine (A) and lindane (L) in subtoxic concentrations (0.07-0.35  $\mu\text{mol}$ ) decreased markedly, but with different intensity: (a) conversion of testosterone (T) to active metabolites (under the influence of  $5\alpha$ -reductase and  $3\alpha$ - and  $17\beta$ -hydroxysteroid dehydrogenase activities) in rat prostate (A 5-23%, L 9-41%) and pituitary (A 4-21%, L 11-35%), in pig pituitary (A 2-14%); (b) formation of specific  $5\alpha$ -dihydrotestosterone (DHT)-receptor complex in rat prostate (A 10-48%, L 13-44%) and (c) formation of estradiol-receptor complex in rat uterus (A 14-41%, L 11-41%). *In vivo* experiments on rat (p.o. 30 mg/kg body weight/daily 7 days) confirmed results received *in vitro*. Enzyme activities for T conversion are expressed as pg of steroid/mg tissues after incubation of fresh tissue with  $^{14}\text{C}$ -T followed by autoradiography. Formation of specific  $^3\text{H}$ -DHT-receptor and  $^3\text{H}$ -estradiol-receptor complexes are measured by Scatchard analysis.

*Key words:* pesticides, reproduction, steroid hormones,  $5\alpha$ -reductase, hormone-receptor complex

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## CELL CULTIVATION AND BIOMASS PRODUCTION: MODEL OF BHK-21 CELLS FOR VIROLOGICAL AND TOXICOLOGICAL APPLICATIONS

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Animal cell technology has become an important branch of Biochemical Engineering since it proved to be the unique way to obtain products applicable in veterinary and human medicine, either as vaccines, therapeutic or diagnostic media. BHK-21 C13 cell line in suspension culture is used as a model. The cultivation of cells from the initial cell bank (ATCC) quantity to the desired scale is carried out in a specifically designed culture medium. The production of the BHK-derived cells starts with cultivation in low cell density T flasks and cell-spin bioreactors. Several cell-spin bioreactors are then combined to inoculate BR-06™ bioreactor specifically designed for cell cultures which require gentle stirring and constant suspension. Bioreactor BR-06™ is equipped with four electronically controlled vertical poles producing a rotating magnetic field as well as with the automatic oxygen and pH control. The controlled and monitored culture parameters are: oxygen reaction, pH, temperature, agitation speed, glucose concentration, cell density, viability and sterility. The cells are cultivated using batch or semi-continuous batch procedure. The cells in the late logarithmic or early pre-confluent phase of growth are selected to give the highest possible initial viability for the freezing procedure. The growth behavior of BHK-21 C13 cells in suspension was as follows: maximum growth rate  $m_{max} = 0.032$ , viability 75-95%, population doubling time  $t_d/h = 26.41$  and maximum cell density  $1.48 \times 10^6$ . The harmful effects of chemicals on ecosystems depend on their entry, distribution, bioaccumulation, binding and degradation. Laboratories provide best conditions for quantification of the effects of agents such as pesticides or toxins on the entire organisms and cellular functions. BHK cells are particularly useful for application in virology. Their recent application in toxicology relates to detection of carcinogenic drugs, cytotoxicity of different organic or inorganic compounds (such as warfarin, diomycin, tunicamycin, staurosporine and zinc transporter proteins), or to monitoring of activated proto-oncogenes in chemically transformed BHK cells.

*Key words:* BHK-21 C13 cells, toxicology, bioreactor, cell application

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## THE USE OF ANIMAL CELLS IN TOXICOLOGICAL RESEARCH *in vitro*

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**B**iological data and real work experience support the proposition that, generally speaking, responses in experimental animals can predict potential health effects in humans. However, species differences in the response to chemicals can be handled easily. Rabbits, dogs and monkeys are used quite rarely now, but are still necessary to identify some types of toxicity. They are also more closely related to humans and thus may be more accurate predictors of human exposure. In general, a varied response in experimental animals - effects at more than one site or more than one species or strain, with increasing response to increasing exposure - provides more convincing evidence of possible human effects than does a response limited to single species or gender. However, the public opinion and recently issued acts on animal welfare recommend or require avoiding unnecessary and groundless pain, anxiety, stress and death of animals. Although studies with animals give information on the reaction of the whole organism, the study of cells *in vitro* has its own advantages. So far, more than 500 *in vitro* test protocols with mammalian cells using various endpoints have been published. However, only a few contribute to reduce the number of animals when applied for screening purposes. The reason for this situation is the lack of validation of the methods. Test protocols developed by one laboratory should generate comparable data in other laboratories. Different laboratories should establish and standardize reproducible cell type characteristics and culture conditions. By now, only a few *in vitro* genotoxicity test protocols have met these demands. Further investigations of possible *in vitro* application of mammal cells in toxicological research pay special attention to hepatotoxicity, nephrotoxicity, neurotoxicity, immunotoxicity, phototoxicity, dermatotoxicity, cell transformation, cytotoxicity and genotoxicity. Furthermore, while *in vitro* reprotoxicity and teratotoxicity are important research fields, the results of broad scale research with cell cultures are still quite limited due to a very a recent start. We anticipate that the future will bring a substantial increase of *in vitro* test protocols concerning mammal cell cultures.

*Key words:* toxicology, animal cells, *in vitro*

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## INTERINDIVIDUAL DIFFERENCES OF ORGANIC SOLVENTS IN TOXICOKINETICS

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Substantial evidence indicates that interindividual variations of several organic solvents in metabolic activity of men may grossly exceed factor 10. For illustration, two populations were found during exposure to methyl chloride: a minority of "poor converters" and a majority of "converters". The differences may be particularly important in exposure to solvents which are hardly metabolized. In many cases, evidence has been obtained that genetic polymorphism is, at least in part, the basis of this variability. Other individual factors could also be important, such as protein binding, renal function, urine flow rates or body-build. A number of physiological changes related to aging are known to contribute to alterations in kinetics, such as increase of relative body mass, reduction of lean body mass, total body water, cardiac output, apparent liver blood flow, renal plasma and globular filtration rate. Sex differences in toxicokinetics were observed for solvents such as toluene, acetone, trichloroethylene and ethanol.

*Key words:* interindividual differences, toxicokinetics, organic solvents, genetic and physiological factors

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## MARKERS OF HEAVY METAL REPRODUCTIVE EFFECTS IN FEMALE RATS

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**M**ore data on the reproductive and perinatal toxicity of both lead and cadmium are still needed. Our experimental studies conducted in laboratories in Croatia and in the USA on female albino rats (Wistar or Sprague-Dawley) have resulted with the following findings. Subchronical oral exposure to lead (3.500 to 7.500 ppm) or cadmium (50 ppm) during pregnancy and lactation caused decreased pup viability (lead), and decreased pup body weight (both lead and cadmium exposure). Serum progesterone concentration in dams exposed orally to cadmium (5.0 ppm) were depressed at the term. Acute *in vivo* exposure of cycling (proestrous) rats to cadmium (5 mg/kg body weight subcutaneously) suppressed steroid production by cultured ovaries. When ovaries from proestrous rats were cultured in 450 micromole cadmium concentration, there was a 50% reduction in progesterone production. Additional *in vitro* studies have shown that the probable site of cadmium action in the steroidogenic pathway is prior to the cholesterol side chain cleavage enzyme.

To conclude, by using these biomarkers of effect, we obtained new data on the effects of heavy metals on female reproduction.

*Key words:* cadmium, lead, reproduction, steroidogenesis, female rats

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## BIOCHEMICAL ASPECTS OF BIOLOGICAL EFFECTS OF PESTICIDES

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**P**esticides are chemicals with diverse chemical structures and modes of action on both pests and humans, having only in common their intended use to kill or repel pests. Beside drugs, pesticides are the most extensively studied chemicals. However, the mode of adverse effects in humans is still not fully understood for the majority of them.

Anticholinesterases, the most frequently used group, are among the most powerful poisons, being incriminated in the majority of cases of human poisoning. Their mode of action, through acetylcholinesterase inhibition, is very well understood and adverse effects can be effectively counteracted by specific antidotes.

Like anticholinesterases, most insecticides affect the nervous system, either by impairing the sodium channel function (pyrethroids, or some chlorinated hydrocarbons) or by interacting with GABA receptors (cyclodienes or fiproles).

Some insecticides act by uncoupling mitochondrial respiration (pirate), or disturbing energy production (cyanides). Insect growth regulators inhibit polymerase, an enzyme crucial for chitin synthesis necessary for the insect growth. Many herbicides interfere with chlorophyll function in the photolysis of water, a mechanism which is irrelevant to mammals. The toxicity of such herbicides for humans is very low. The carcinogenic potential of pesticides is rarely encountered, and any chemical with such an action is soon abandoned as a pesticide.

*Key words:* anticholinesterase, insecticide, carcinogenic potential

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## CADMIUM-INTOXICATED RATS MANIFEST DERANGED STRUCTURE OF MICROTUBULES IN LIVER AND KIDNEY CELLS

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Chronic cadmium (Cd) intoxication in man and experimental animals is manifested by defects in reabsorptive and secretory functions of the renal proximal tubule. Polyuria, glucosuria, aminoaciduria, phosphaturia, and proteinuria are common signs of Cd nephrotoxicity whose mechanisms are poorly understood at the cellular level. In the cell, Cd may affect microtubules whose arrangement and function are responsible for vesicle flow between the plasma membrane proximal tubule and intracellular organelles (endo- and exocytosis). An impaired endo- and exocytosis due to microtubule derangement may lead to proteinuria and diminished cellular uptake of solutes due to a loss of specific transporters in the plasma membrane. We studied endocytosis and microtubules in the liver and kidney cells in control and Cd-treated rats (2 mg Cd/kg b.w., s.c., daily for 14 days). As measured by the atomic absorption spectrometry, the content of Cd in tissues increased with time, after 14 days being around 250 and 400 mg/g wet weight in the kidney cortex and liver, respectively. To test the cell endocytosis, rats were injected *in vivo* with the fluorescein isothiocyanate-dextran (FITC-dextran, 35 mg/rat, iv). Compared to controls, in both liver and kidney proximal tubule cells, Cd-intoxicated rats exhibited a much lower uptake of FITC-dextran due to an impaired endocytosis. Using the monoclonal anti-tubulin antibody, the immunofluorescence studies showed a bundle-like arrangement of cell microtubules in frozen control tissue sections. In tissues from Cd-treated rats, the arrangement of microtubules was irregular, indicating that the recycling of intracellular vesicles, the amount of several brush-border membrane proteins, including the proton-ATPase, carbonic-anhydrase, and dipeptidyl-peptidase IV were drastically reduced in Cd-treated rats. Thus, Cd inhibits cell endocytosis and depletes plasma membrane of the important proteins possibly by interfering with the microtubule-dependent intracellular vesicle flow.

*Key words:* cadmium, microtubules, nephrotoxicity, liver, kidney

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## THE INFLUENCE OF LINDANE AND MALATHION™ ON ANDROGEN MECHANISMS IN RAT PROSTATE

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The  $5\alpha$ -reduction of testosterone and the formation of specific  $5\alpha$ -dihydrotestosterone-receptor (DHT-R) complex are the prerequisites for androgen action in target cells. Due to their chemical properties, various xenobiotics can accumulate in tissues and interfere with these biochemical processes within the cell. The influence of lindane and Malathion™ on the conversion of testosterone into DHT, and on the binding of DHT to its specific receptors, were studied in the rat prostate. Although the conversion into DHT did not change immediately after the cessation of lindane treatment (po 60 mg kg<sup>-1</sup> daily, 7 days), it lowered for 57.2% on the seventh post-treatment day. Both lindane and Malathion™ inhibited the  $5\alpha$ -reduction of testosterone *in vitro* significantly (up to 42.3% and 28.2%, respectively). Lindane exerted *in vivo* and *in vitro* a strong inhibitory effect on the formation of DHT-R complex in rat prostate cytosol. The inhibition was characterized as fully noncompetitive. The results suggested that the lowered complex formation was the consequence of conformational changes in the receptor molecule caused by lindane, rather than the effect of altered testosterone metabolism. Malathion™ was also found to be a strong inhibitor of prostatic DHT-R complex formation *in vitro*. Lindane and Malathion™ in the equimolar mixture exerted synergistic effect on the formation of androgen-receptor complex.

*Key words:* lindane, Malathion™, testosterone metabolism,  $5\alpha$ -dihydrotestosterone-receptor

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SECTION 4

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# Genotoxicology





## ANTIMUTAGENS AND ANTICARCINOGENS IN FOOD

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The data accumulated from epidemiological and experimental studies over the last decade indicate that vegetables, cereals and fruit may contain cancer-preventing components. The fact that most of them are antioxidants may explain their cancer-preventing activity. A large number of compounds, belonging to more than 20 different classes, show chemopreventive potential. The dietary anticarcinogens can be divided into nutritive and non-nutritive compounds.

The antimutagenic effect of fermented milk interacting with immediate mutagens sodium azide and 4-nitroquinoline-N-oxide was investigated using 4 strains of *Salmonella typhimurium* (TA100, TA98, TA1535 and TA1538). The mutagenic activity of 4-nitroquinoline-N-oxide decreased in all strains, while the mutagenic activity of sodium azide decreased in strains TA100 and TA1535.

*Key words:* antimutagens, anticarcinogens, food, Ames test

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## GENETICALLY ENGINEERED CELLS IN MUTATION RESEARCH AND TOXICOLOGY

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**I**n order to elucidate the mechanisms protecting mammalian cells from mutagenic and genotoxic effects of alkylating mutagens, we have generated Chinese hamster cells by transfection with cDNA expression vectors that overexpress human genes involved either in DNA repair, damage tolerance or detoxification. Among these are O<sup>6</sup>-methylguanine-DNA methyltransferase (MGMT), N-methylpurine-DNA glycosylase (MPG), apurinic endonuclease, poly(ADP)ribose polymerase, metallothionein IIA, polymerase  $\beta$  and a factor supposed to be involved in alkylation tolerance. Furthermore, transgenic mice were generated that express human MGMT in their skin. Cells overexpressing MGMT were highly protected from the mutagenic, toxic, recombinogenic and carcinogenic activity of methylating agents. They also proved to be protected from antineoplastic drugs that are derivatives of nitrosourea. From these results conclusions will be drawn as to the critical lesions and mechanisms leading to gene mutations, chromosomal aberrations, sister chromatid exchanges, cell death and malign transformation.

*Key words:* DNA repair, mutagenesis, gene expression

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## GENOTOXIC EFFECT OF HERBICIDE “DICURAN”

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Selective herbicide “Dicuran” is widely used for weeding in wheat fields. We used *Allium* test to investigate the effect of “Dicuran” (active ingredient 50% chlortoluron) on wheat seedling (*Triticum aestivum* cv. Pitoma) and shallot root tip cells (*Allium ascalonicum* auct.). The used concentrations of “Dicuran” corresponded to those normally applied in the field. Furthermore, we tested the mutagenicity and cytotoxicity of “Dicuran” on bacterial strains *Salmonella typhimurium* TA100 and TA98. “Dicuran” showed cytotoxic effect on both plants causing inhibition of root growth. This was confirmed after the analysis of mitotic activity in root tip cells. The genotoxicity of the herbicide manifested through numerous chromosome aberrations such as anaphase and telophase bridges, chromosome fragments, laggard chromosomes and micronuclei.

We also noticed mitotic irregularities like c-mitosis, disturbed metaphases and anaphases, as well as mitotic spindle multipolarity. Stickiness was frequently found and it accompanied all mitotic phases. The results obtained with “Dicuran” on two strains of *S. typhimurium* showed mutagenic effect in a base-substitution sensitive strain TA100.

*Key words:* chlortoluron (“Dicuran”), cytotoxicity, mutagenicity

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## EXAMINATION OF MUTATIONS, RECOMBINATIONS AND ANEUPLOIDY CAUSED BY ANTIDOTES

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Over the past years, many mono- and bis-pyridinium aldoximes have been used as antidotes for poisoning with organophosphorus cholinesterase inhibitors (e.g. pesticides, nerve gases). One of them, PAM-2, is a well known and commonly used drug. The compounds PPDO-2 and PPDO-4 (pyridoxal-oxime derivatives) with similar reactivating effect as PAM-2 have been synthesized at our Faculty. We tested on different microbial test-systems for possible genotoxic effects of PPDO-2 and PPDO-4. We used *Salmonella typhimurium* (TA98, TA100, TA102 and TA104) with and without metabolic activation to detect the frameshift and base substitution mutations. At the same time, we chose yeast *Saccharomyces cerevisiae* as the eukaryotic test system to evaluate the induction of mitotic aneuploidy (strain D61.M) by using standard and cold treatments. Strain D7 was used to detect gene conversion. Negative results (which correspond to our previous investigation) strongly suggest the importance of further testing in order to establish potential antidote effect of these substances.

*Key words: genotoxicity, PPDO-2, PPDO-4, Salmonella typhimurium, Saccharomyces cerevisiae*

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## A CASE OF WILSON'S DISEASE

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Wilson's disease results from a defect in the metabolism of copper and is inherited as an autosomal recessive trait. It is a rare disease, 3-6 cases per 100,000 people, manifesting at the age between 6 and 60. Copper accumulates in various tissues and organs, most frequently in the liver, basal ganglia, kidneys and cornea.

Determination of tissue copper concentration is important for the diagnosis of Wilson's disease. This study presents the tissue analysis of a patient with Wilson's disease. The patient died from sepsis during the treatment of fire burns.

The tissue samples were obtained from autopsy. The control samples were taken from autopsy of two healthy subjects killed in an accident. The presence of copper in the tissues was determined by line spectrum-based mass spectrography, and its concentration by atomic absorption. The tissue samples were analyzed by standard histologic staining, and the presence of copper was histochemically tested.

Histochemical staining failed to demonstrate the presence of copper which was qualitatively proved by mass spectrography. The following copper concentrations were quantitatively determined by the method of atomic absorption: liver 240  $\mu\text{g/g}$  (control: 21  $\mu\text{g/g}$ ), brain 73.8  $\mu\text{g/g}$ , kidney 30  $\mu\text{g/g}$ , pancreas 53.8  $\mu\text{g/g}$  and spleen 8.8  $\mu\text{g/g}$ .

This study verified the Wilson's disease by determining the increase in tissue copper concentrations. The obtained results contribute to the understanding of this rare disease.

*Key words:* Wilson's disease, copper, mass spectrography, atomic absorption, histochemistry

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## THE EFFECT OF OVEREXPRESSION OF *alkA* AND *tag* GENES ON SURVIVAL AND MUTATION INDUCTION IN *Salmonella typhimurium*

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Constitutively expressed repair enzyme 3-methyladenine DNA glycosylase I encoded by the *E. coli tag* gene removes N<sup>3</sup>-methyladenine from DNA by glycosylic cleavage in the first step of the base excision repair. Enzyme 3-methyladenine DNA glycosylase II, encoded by the *E. coli alkA* gene, removes alkyl groups from the N<sup>3</sup> and N<sup>7</sup> positions of both purines and from the O<sup>2</sup> position of both pyrimidines. Since *Salmonella typhimurium* harbours only a constitutively expressed glycosylase functions, it implies that *S. typhimurium* is phenotypically AlkA<sup>-</sup>. In order to investigate the effect of the overexpressed *alkA* and *tag* genes on survival capacity and possible modifications in mutation induction and thereby elucidating the importance of 3-methyladenine and 3-methylguanine in cellular sensitivity to methylating agents, we transformed the *S. typhimurium* hisG46, TA1535, hisG428 and TA101 strains with plasmids carrying either *alkA* (pYN1000) or *tag* (pCY5) with their corresponding regulatory regions. The overexpression of AlkA could not completely complement AlkA<sup>-</sup> phenotype in any of the tested strains, but the level of suppression in the *uvrB*<sup>-</sup> strains was about 20% higher than in the isogenous *uvrB*<sup>+</sup> strains. However, the overexpression of the *tag* gene completely suppressed AlkA<sup>-</sup> phenotype in all experimental strains. In all tested strains the number of revertants (his<sup>-</sup>→his<sup>+</sup>) decreased to the level of spontaneous revertants. The decrease in the level of bi-directional transitions in *S. typhimurium* (GC→AT and AT→GC) to the spontaneous level clearly showed that in those prokaryotic systems, similar to *E. coli*, 3-methylguanine represents the second most important toxic and mutagenic lesion.

*Key words:* the Ames strains, *alkA/tag* genes, base excision repair

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SECTION 5

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# Clinical Toxicology





## RED BLOOD CELL GLYCEROL HAEMOLYSIS AS AN EFFECT BIOMARKER OF HAEMATOLOGIC DAMAGE IN OCCUPATIONAL LOW-LEVEL BENZENE EXPOSURE

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**B**enzene, a myelotoxin and leukemogen, is often present in the working and living environment, and it is therefore important to assess the most suitable effect biomarker of low-level benzene exposure (1, 2, 3).

Forty-nine female workers in a shoe factory exposed to solvent mixture and twenty-seven non-exposed controls were investigated. The concentrations of benzene in the workshop atmosphere, the levels of benzene in blood and phenols in urine, before and after work, confirmed occupational exposure to benzene. Complete blood cell count with differential, haemoglobin, haematocrit, erythrocyte indices, reticulocytes, serum iron and red blood cell glycerol haemolysis time (GLT<sub>50</sub>) were determined in all subjects. Data were analysed by Student t-test and Spearman correlation.

The average concentration of airborne benzene in shoe manufacturing process was 5.55 (range 1.9-14.7) ppm. Although there was no evidence of clinically manifest haematologic impairment, lower hemoglobin level ( $P < 0.02$ ) and mean corpuscular haemoglobin concentration ( $P < 0.01$ ), higher mean corpuscular volume ( $P < 0.03$ ), band neutrophils ( $P < 0.001$ ) and GLT<sub>50</sub> ( $P < 0.000001$ ) were detected in the shoe workers as compared to controls. GLT<sub>50</sub> correlated with benzene exposure time, benzene in blood and phenol in post-shift urine as significant benzene exposure biomarkers.

In comparison with other haematologic parameters indicative of benzene induced health effects, GLT<sub>50</sub> correlated with the largest number of exposure biomarkers. Thus GLT<sub>50</sub> appears to be an acceptable effect biomarker in low-level benzene exposure.

*Key words:* benzene, glycerol haemolysis time, haematotoxicity

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## ACUTE CYANIDE POISONING: CLINICAL SPECTRUM, DIAGNOSIS AND TREATMENT

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Cyanide poisoning, more common than generally appreciated, may take many forms. Industrial intoxications occur due to the extensive use of hydrogen cyanide and cyanide salts as reaction products. Domestic poisoning involving cyanide most often occur in the complex setting of smoke inhalation. Suicidal cyanide ingestions are rare, but often dramatic. Cyanogenic compounds, such as cassava and the nitriles, may produce acute or delayed intoxication, depending on dose and metabolism. Clinical signs of poisoning include headache, vertigo, agitation, confusion, followed by coma, convulsions, and death. Laboratory confirmation of cyanide in body fluids is generally delayed. In the appropriate setting, anion gap metabolic acidosis and elevated plasma lactate, associated with cardiovascular collapse, should evoke cyanide intoxication. Immediate treatment of cyanide poisoning includes 100% oxygen, assisted ventilation, decontamination, correction of acidosis, and support of blood pressure. First line antidotal therapies include oxygen, hydroxocobalamin, di-cobalt EDTA, and methemoglobin-inducing agents. Hydroxocobalamin is an attractive antidote due to its rapid binding of cyanide and its lack of serious side effects, even when administered in the absence of cyanide intoxication. Sodium thiosulfate is useful for replenishment of depleted rhodanese stores, but its effect is slower than with other antidotes. It is most useful as an adjunct in the treatment of acute cyanide poisoning, as well as in the subacute intoxications with cyanogens. The evaluation of treatment efficacy is primarily based on correction of hypotension and reversal of lactic acidosis. However the final prognosis depends on the degree of permanent central nervous system injury.

*Key words:* cyanide, cyanogens, acute poisoning, smoke inhalation, antidotes

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## COLCHICINE-INDUCED MULTISYSTEM ORGAN FAILURE MARKEDLY ALTERS ITS KINETICS

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**T**hough relatively rare, colchicine poisoning remains associated with high morbidity and mortality. While a new treatment modality in the form of colchicine-specific Fab fragments is only emerging, currently available therapy remains largely supportive. The elimination of colchicine is considered to occur primarily by hepatic metabolism, following a first-order process, with significant biliary excretion and enterohepatic circulation. Renal excretion is responsible for approximately 20% of colchicine elimination. We report here an intoxication in which the initial manifestation was relatively benign, but for which the subsequent course was marked by anuric renal failure, hepatic insufficiency, disseminated intravascular coagulation defect, biliary stasis, and death. Serum colchicine concentrations remained quasi-constant over three days of the patient's struggle for life, and consistent with the marked alterations in metabolism and excretion. Severe colchicine toxicity, resulting in a vicious cycle of progressive organ dysfunction and impaired elimination, appears to offer the best explanation of our findings. The ingested macrolide antibiotic josamycine was likely to play an important role in reducing the biliary excretion of the colchicine. The potential for such an outcome serves as a reminder of the need for an early aggressive therapy, careful evaluation of potential medication interactions, and consideration of immunotherapy where available.

*Key words:* colchicine, acute poisoning, multisystem organ failure, toxicokinetics, metabolism

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## TOLERANCE OF HIGH DOSE HYDROXOCOBALAMIN IN VICTIMS OF SMOKE INHALATION

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**H**ydroxocobalamin (OHCO) has been proposed as a cyanide antidote in smoke inhalation due to its lack of significant side effects. We studied the clinical and biological tolerance of a high dose (5-15 g) intravenous OHCO in human victims of smoke inhalation.

Prospective, open-label, uncontrolled study. Victims of smoke inhalation with soot in the mouth and altered consciousness were treated with OHCO 5g over 20 min at the fire scene after blood sampling for cyanide and carbon monoxide. Repeated doses were permitted up to a maximum of 15 g. Systolic blood pressure, heart rate, neurologic condition and adverse effects (rash, bronchospasm, and sphygmomanometry) were monitored. Laboratory criteria included blood cyanide and carbon monoxide concentrations, plasma lactate, glucose, complete blood count, creatinine, CPK and liver function tests. Patients were monitored over three days for laboratory and clinical effects.

A total of 69 patients, 33 male and 36 female, were admitted for treatment. 37 patients were in coma, 27 suffered initial loss of consciousness, 14 patients suffered initial cardiac arrest. Median dose of OHCO was 5 g. 46 patients had complete resolution of neurologic disturbances, 13 suffered decerebration, and 9 had persistent neuropsychiatric sequelae. 13 patients died by decerebration, 6 from delayed complications. No significant changes in heart rate, blood pressure, or adverse effects related to OHCO were observed. Cyanide intoxication (blood concentration  $\geq 39$   $\mu\text{mol/L}$ ) was confirmed in 42 patients. Median carbon monoxide level was 2.4 mmol/L. Transient renal and liver dysfunction was observed only in patients suffering from cardiac arrest. No other significant laboratory abnormalities were observed. Hydroxocobalamin appears to be safe in the treatment of smoke inhalation victims.

*Key words:* hydroxocobalamin, smoke inhalation, cyanide, carbon monoxide, antidotes

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## PRE-HOSPITAL EMERGENCY CARE IN ACUTE POISONING

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**T**reatment of acute poisoning "on the spot" in urgent and dramatic situations requires a specially trained clinical toxicologist for pre-hospital care. The Emergency Medical Service personnel, who are usually the first to treat acutely poisoned individuals, often have an extremely important role in the chain of events. During their first contact with the physician, approximately 75% of patients show mild or no symptoms at all and do not appear vitally endangered. Acutely poisoned patients with manifest symptoms, or those whose health or life is threatened, must be treated according to a precisely determined plan.

The diagnosis is based on anamnestic and/or heteroanamnestic data and indirect evidence (packing or leftovers of poison). The patient's symptoms and signs may confirm the suspected poisoning, but are rarely sufficiently typical to enable accurate diagnosis. Where possible, further absorption of poison should be prevented during the pre-hospital treatment, depending on the way of consumption. After removal from the immediate source of poisoning, cardiopulmonary resuscitation can be performed in accordance with conventional guidelines. It is obligatory to monitor the consciousness, respiration, circulation and body temperature of the patient. In case the monitored parameters show deterioration, symptomatic and supportive treatment should be implemented. There are few cases when specific antidotes can be used: atropine sulfate (organophosphorus insecticides), naloxon (opioids), flumazenyl (benzodiazepines), dicobalt edetate (cyanides) and oxygen (carbon monoxide).

Prevention of further absorption of poison, cardiopulmonary resuscitation, symptomatic and supportive treatment and seldom the application of antidotes sum up the basic approach to the acutely poisoned patient in pre-hospital care.

*Key words:* acute poisoning, pre-hospital emergency care

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## NARCOTICS RELATED DEATHS IN SPLIT, 1995

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Since the beginning of the war in Croatia, an increase in the number of drug addicts and fatal intoxication has been encountered in the Split region. Narcotics related fatalities (n=24) were prospectively investigated over a 12-month period (1995). The conclusions regarding the cause of death have been based on information from police reports, autopsy findings and toxicological results. The main narcotic involved in drug addict deaths was heroin, while others were rarely found. Blood alcohol level was analysed for all cases. The additional alcohol consumption increased the risk of heroin overdose.

*Key words:* drug addict, heroin, autopsy, toxicology

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## INTOXICATION INDUCED COMATOSE STATES AND THE RISK OF MALIGNANT ARRHYTHMIA

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Comatose patients, 27 intoxicated, 30 with stroke (cerebrovascular accident) and 30 control were analyzed for Q-T interval (Q-T<sub>c</sub>) using Bazett's formula. Relative risk of prolonged Q-T<sub>c</sub> was calculated for each group. Q-T<sub>c</sub> was prolonged in 56.6% of intoxicated patients, in 53.3% of patients with stroke and in 6.7% of controls. The relative risk of prolonged Q-T<sub>c</sub> for intoxicated patients was 17.5, while for patients with stroke it was 16.0. There was very little difference between the two groups: 0.9. The highest values of Q-T dispersion were seen in intoxicated patients: 88.9±51.8 ms, as compared to patients with stroke: 75.6±43.0, and controls: 31.4±10.0 ms.

There was no correlation between Q-T<sub>c</sub> and serum potassium level, serum magnesium or calcium level, or metabolic acidosis.

*Key words:* coma, intoxication, stroke, Q-T interval, relative risk

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## MYOTOXICITY IN LIPID- LOWERING THERAPY

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**M**uscular syndrome is a rare, but typical adverse effect of lipid-lowering therapy, particularly with fibrates. It consists of myalgia, malaise, muscle tenderness and elevated serum activity of muscle enzymes such as creatine kinase, lactic dehydrogenase and aspartate aminotransferase. Light microscopy reveals atrophy, vacuolization and degeneration of muscle fibres. Ultrastructural investigations show degenerative changes with damaged neuromuscular junctions. An impairment of fatty acid oxidation is probably the leading mechanism, followed by the disturbance of energy transfer in mitochondria. Although the muscular toxicity can be shown in rats, the model is difficult to monitor. Our observations stress the importance of the plasma level of the drug involved and provide some key elements for treatment. In the recent years, it has been observed that the drug combinations (e.g. gemfibrozil and lovastatin, or lovastatin and cyclosporin) have a potentiating effect on myotoxicity. Possible mechanisms are further discussed.

*Key words:* myotoxicity, lipid-lowering, humans, rats

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## POISONING WITH DAPSONE

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**S**ignificant incidence of dapsone poisoning has been reported in Asia, and a case of a 13-year-old girl poisoned with dapsone represents medical curiosity in Croatia. Dapsone is generally used for treatment of leprosy, its resorption from alimentary tract being very good. Half-life of dapsone is 48 hours and 50% binds to plasma proteins. It is metabolized in liver by acetylation and N-oxidation and partially enters enterohepatic circulation. Excretion is by urine, only 10-20%, in unmetabolized form. Therapeutic doses are 1-2 mg/kg for children, up to 500 mg for adults. Intoxication is present at ingestion of 1.5 g or more. Most usual signs of acute poisoning are: sickness, vomiting, abdominal pain, cyanosis, methaemoglobinemia, haemolytic anaemia, jaundice and coma. The possible consequence of poisoning is peripheral motor neuropathy.

The girl was hospitalized 18 hours after ingesting 2-3 grams of dapsone in a suicide attempt. She had all symptoms of acute poisoning except for coma. The level of methaemoglobin at arrival was 44% (normal 0.5-1.5%), and was normalized after twelve days. Jaundice was spotted on the fourth day of hospitalization with the highest level of bilirubin at 143  $\mu\text{mol/L}$  (normal 4-20  $\mu\text{mol/L}$ ). The lowest level of haemoglobin in blood was recorded on the tenth day (77 g/L). The detoxification included two exchange transfusions and five plasmaphereses, administration of activated charcoal, cathartics and forced diuresis. In addition, we used oxygen therapy and ascorbic acid. Because G-6-PDH activity was normal, methylene blue was applied intravenously.

Unfortunately, the girl developed peripheral, mostly motor, neuropathy as a consequence of dapsone poisoning.

*Key words:* dapsone poisoning, peripheral motor neuropathy

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## CHILDREN POISONING IN A FIVE YEAR PERIOD - AN OVERVIEW

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**D**uring a five year period (1990-1994), 257 (2%) children (out of 13,692) were hospitalized due to poisoning, 56.4% of which were boys and 43.5% girls. Diagnoses were established on the basis of anamnesis, clinical findings and laboratory results. Poisoning was most frequent in the age group between 1 and 3 years (31.5%), while in other two age groups (4-5 and 6-7 years) the incidence was halved (14.5% and 14.1%). One third of the children were in the age group between 8 and 14 years. The study includes mushroom poisoning, as a part of the Department's responsibilities, which was highly represented (28.8%) in the overall distribution. The same incidence was registered for the drug poisoning (28.8%), most frequently by psychotropic drugs (40.1%). It is important to point out that the very low incidence of alimentary intoxications in our research is due to the fact that these cases are usually referred to institutions for infectious diseases. Other causes of poisoning were alcohol (8.9%) and cleaning substances (6.6%). Average hospitalization lasted for 6.15 days, while the mushroom poisoning had the longest average hospitalization (7 days). Looking at the distribution of poisoning by seasons, mushroom poisoning was most frequent in autumn, while other poisonings (drugs, oil-products and cleaning substances) were distributed equally throughout the year. 55.3% of treated children recovered completely, 44.2% partially, while 3 children died (1 poisoned with organophosphate and 2 with mushrooms).

*Key words:* children poisoning

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## THE FIRST SEVERE CASE OF PARACETAMOL POISONING IN CROATIA

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**T**here are thousands of cases of paracetamol poisoning in adults and children described in literature with many consequent deaths. In 1991, only USA reported about 77,000 poisonings and 82 deaths for all age groups. There has been no reports of paracetamol poisoning in Croatia so far. We have an unofficial knowledge of about 25 persons of all age groups who received a significantly larger dose of paracetamol, but did lacked any clinical or laboratory findings of poisoning. A seventeen-month-old girl was treated with paracetamol and antibiotics at home, after which she was hospitalized for five days with the indication of fever. Antibiotic treatment continued due to urinary tract infection. From the third day of hospitalization on she was afebrile. That same day she was vomiting and displaying bad general condition and metabolic acidosis. Her condition improved the next day, but the day after it deteriorated again. Clinical findings were those of sopor, hepatomegaly and jaundice. The girl was transported to our unit as suspect of Reye's syndrome. Laboratory results were as follows: AST activity was 6200, ALT 4283 U/L, prothrombin index <0.1. The unknown etiology of hepatic insufficiency with encephalopathy inspired us to perform urine toxicological analysis. As a result, we found 205.9 µmol/L of paracetamol. Serum concentration of paracetamol was 9 µmol/L. Interpolation in Rumack-Matthews nomogram pointed out substantial paracetamol poisoning. Since more than 24 hours passed from the ingestion, we decided not to perform a specific treatment with N-acetylcysteine. Instead, we treated her with the fresh frozen plasma for seven days. Within 30 hours from admission to our unit, the girl was conscious, and not displaying hepatomegaly and jaundice. The subsequent normalization of serum transaminase activity occurred within ten days. The girl was discharged after three weeks in good condition. Two months later, the prothrombin index was normal.

*Key words:* paracetamol, poisoning, Croatia

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RISK PROFILE OF LIPID  
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**M**onitoring plasma levels of cholesterol and triglycerides has become a standard medical practice since high cholesterol levels are an important risk factor for development of arteriosclerosis. When diet alone does not decrease cholesterol levels below 5.2 mmol/L, drug treatment with bile sequestrants, nicotinic acid, fibrates or the more recently developed statins is used. Unless in excessive doses, all these drugs are well tolerated. The most common side effects are given in the table below:

Bile sequestr.	Nicotinic acid	Fibrates	Statins	
Meteorism	Headache, "Flush"	Gastric upset	Constipation	Side effects
Nausea, Pruritus Constipation		Nausea Headache	Nausea Headache	
Steatorrhea	Panic attacks	Muscular cramps	Myalgia, Rhabdom.	spec. side
Acidosis	Liver failure Loss of vision	Hepatitis (?) Bile concr. (Clop)	Insomnia Cataracts (?)	effects

Animal experiments revealed some side effects which epidemiological surveys did not note in humans. These as well as clinical side effects like sleep disorders, myotoxicity, violent deaths, etc. will be discussed in detail. An immediate risk/benefit evaluation is not possible since the patient does not feel immediate consequences, which are visible only in the long run and after a continuous treatment. However, the benefit from proper monitoring of patients belonging to a risk group, such as people with kidney impairment, is outweighing the risks by far.

*Key words:* adverse effects, lipid-lowering therapy, humans, animals

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## PHARMACOKINETICS OF HYDROXOCOBALAMIN IN VICTIMS OF SMOKE INHALATION

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**H**ydroxocobalamin (OHCO) has been proposed as a cyanide antidote. Little is known, however, about its pharmacokinetics in human cyanide poisoning.

We prospectively studied the pharmacokinetics of OHCO in 11 smoke inhalation victims, all of whom but one showed objective evidence of cyanide exposure. We followed the serum OHCO levels from the moment immediately before administering a single 5-gram dose of hydroxocobalamin until the 6th day upon that event.

The results (mean  $\pm$  standard error) suggest a two compartment model. Distribution half-life was on the order of  $1.86 \pm 0.34$  hours and the elimination half-life  $26.2 \pm 2.7$  hours. The apparent volume of distribution was  $0.45 \pm 0.03$  L/kg. Renal and total body clearance were  $0.31 \pm 0.06$  and  $0.83 \pm 0.07$  L/h, respectively.

The apparent volume of distribution suggests a predominantly extracellular partitioning of the antidote, even in the presence of cyanide, an important factor in terms of its antidotal effect. Hydroxocobalamin's elimination half-life in these cyanide-exposed patients exceeds by far those found in previous studies on dogs and minimally-exposed humans. If confirmed, this half-life suggests that a single dose of OHCO, sufficient to bind the present cyanide, should be adequate.

*Key words:* hydroxocobalamin, cyanide, smoke inhalation, pharmacokinetics, antidotes

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## THE ROLE OF EXTRACORPOREAL CIRCULATION IN THE TREATMENT OF HERBICIDE POISONING (PARAQUAT)

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**P**araquat poisoning is usually a result of a suicide attempt, and is rarely encountered in clinical practice. Paraquat accumulates selectively in the lung, causing severe pulmonary edema and fibrosis. Progressive respiratory insufficiency anticipates a fatal outcome. During the last 15 years, we treated 5 patients who attempted suicide by taking a large dose of paraquat (quite over the estimated lethal dose). In addition to usual measures (activated charcoal, saline cathartic), all patients were treated by haemodialysis and/or haemoperfusion. The first patient who died, was treated by haemodialysis, only when acute renal failure developed. The others were treated by haemodialysis and haemoperfusion immediately after admission. Procedures were repeated daily for 5-7 days, and all four patients survived. Though the literature data are inconclusive, early treatment by haemodialysis and haemoperfusion greatly improved survival for our patients.

*Key words:* paraquat, hemodialysis, hemoperfusion

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## TREATMENT OF ACUTE ORGANOPHOSPHATE POISONING

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**A**cute organophosphate poisoning, mainly with ingested insecticides, usually comes as a consequence of an accident or a suicide attempt. Organophosphates cause inhibition of cholinesterase enzymes with the consequent accumulation of endogenous acetylcholine at nerve receptors. The result is either the muscarinic effect with enormous bronchial secretion, or the nicotinic effect manifested by the paralysis of respiratory musculature followed by suspension of breathing or by direct effects on the CNS with depression of respiratory center. Poisonings with organophosphate insecticides are severe and fatal unless treated immediately and adequately. Over a period of 10 years, we treated 23 patients poisoned with organophosphates. The treatment consists of early gastric lavage, administration of atropine in adequate doses and early use of specific antidotes, pralidoxim or obidoxim. Suction of pulmonary secretion, occasionally by endotracheal intubation and artificial respiration is often necessary. During the treatment, we must control the clinical status of the patient and the activity of blood cholinesterase. With adequate intensive care, most patients have a fair chance for total recovery.

*Key words:* organophosphate insecticides, acute poisoning

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## MANAGEMENT OF ACUTE PHENOTIAZINE POISONINGS

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Neuroleptic poisonings are most common in the Intensive Care Unit and mainly concern psychotic patients who attempted suicide by ingesting large doses of medical drugs. Although the exact mechanism of action has not yet been determined, it may be related to antidopaminergic as well as antagonistic effect on alpha-adrenergic, serotonergic, histaminic (H1) and muscarinic receptors. Gastric lavage and activated charcoal are recommended. Monitoring of respiratory and cardiovascular function is mandatory. Peripheral alpha-adrenergic blocking activity causes vasodilatation, hypotension and reflex tachycardia. Compensation of body fluid loss with optional dopamine treatment are recommended therapeutic measures. Ventricular ectopic activity could lead to serious and refractory ventricular tachyarrhythmias; ventricular tachycardia, torsade de pointes and ventricular fibrillation. Lidocain or phenitoin and electroconversion are recommended as well as the acute electrostimulation in case of total AV block. Phenothiazine effects could be counteracted by diphenhydramine and anti-cholinergic effects by physostigmine. Thermoregulation disturbances are possible. Neuroleptic malignant syndrome characterized by hyperthermia and rhabdomyolysis with acute renal failure has a great mortality rate. This work presents our experience.

*Key words:* phenothiazines, poisoning, intensive care

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## DMSA IN THERAPY OF OCCUPATIONAL LEAD POISONING

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**D**MSA (2,3-meso dimercaptosuccinic acid) is a new oral chelating agent. Its efficacy was confirmed in the USA, especially in the treatment of children lead poisoning. There are few studies related to DMSA chelation in occupationally exposed workers. This study introduces DMSA for the first time in our country with intention to evaluate its efficiency in chronic occupational lead poisoning. Seven workers, employed in a lead battery factory for at least four years, with elevated blood lead concentrations (PbB) ( $>50 \mu\text{g}/100 \text{ ml}$ ) and a positive EDTA lead mobilization test ( $>600 \mu\text{g Pb}/24 \text{ h urine}$ ), were treated with DMSA for 19 days. DMSA was administered orally in doses of 700 mg DMSA every 8 hours from day 1 to day 5, and 700 mg every 12 hours from day 6 to day 19 of the treatment. Blood lead values declined during the treatment (mean PbB decreased to 15.4% of pretreatment values) with a simultaneous increase in urinary excretion of lead ( $P < 0.01$ ). However, 15 days after the therapy, PbB values rebounded for 35%, but they did not exceed the threshold level ( $30 \mu\text{g}/100 \text{ ml}$ ). The EDTA lead mobilization test detected a 54% decline of pretreatment values ( $P < 0.01$ ) suggesting a significant reduction of lead in the body.

Our results show that DMSA is an efficient chelating agent for treatment of chronic lead poisoning in occupationally exposed patients.

*Key words:* DMSA treatment, chronic lead poisoning, occupational exposure

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## INVESTIGATION OF COLOR VISION IN WORKERS EXPOSED TO ORGANIC SOLVENT MIXTURES

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We examined the color vision of 40 workers employed in a chemical factory, each day briefly exposed to mixtures of organic solvents. The control group comprised 50 workers employed in an electric cable factory and not occupationally exposed to any organic solvent.

The groups were of similar age, education and length of work service. The weekly amount of consumed alcohol and the smoking index in both groups were comparable.

Qualitative and quantitative components of color vision were tested by Lanthony D-15 desaturation test.

Although Color Confusion Index was higher in the exposed than in the non-exposed group (1.14 vs. 1.11) and an impairment in the blue-yellow range was more frequent in the exposed persons (32.2% vs. 27.7%), the differences between the groups were not statistically significant.

The results of this study indicate that brief exposure to solvent mixtures does not significantly impair color vision.

*Key words:* solvent mixtures, Lanthony D-15 desaturation test, color vision

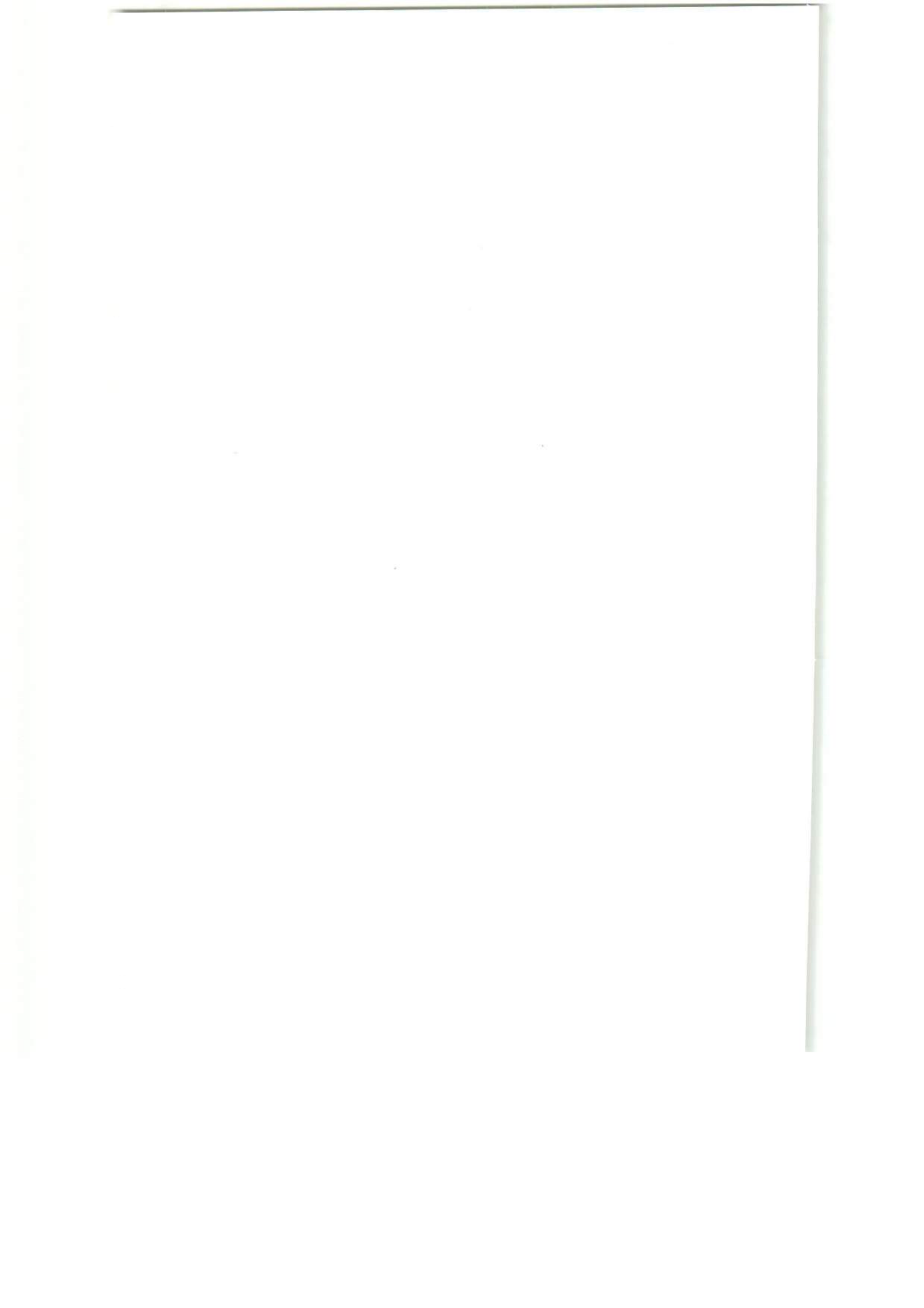
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SECTION 6

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Effects of War on People  
and Environment





## INTERNATIONAL CO-OPERATION IN CHEMICAL DISASTERS

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**M**ajor deficiencies continue to exist in arrangements for dealing with major chemical incidents in all countries. These include lack of community preparedness around hazardous installations, inadequate involvement and training of health professionals in planning and emergency response measures, and the low priority given to research into the human effects of accidental chemical exposures in industry and in the community from toxic releases. International collaborative efforts are demonstrating a new consensus on the role of health professionals (public health and occupational physicians, epidemiologists and toxicologists) in chemical incidents and disasters. A framework is provided by the UN International Decade for Natural Disaster Reduction. Current gaps in the successful management of major incidents include defining susceptibles, the relevance of plume modelling to human exposure (especially in fires), exposure assessment, evacuation criteria, and environmental vulnerability factors.

*Key words:* chemicals, disasters, preparedness, mitigation

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## IMPORTANCE OF TOXICOLOGICAL DATA FOR TOXICOLOGY SERVICE IN CROATIA AS AN ORGANIZED RESPONSE TO ACCIDENTS

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**A** key to successful solution of any problem is to possess appropriate data and information. The same goes for toxicology, particularly in situations requiring prompt reaction, such as accidents.

Data collecting is a very complex and multidisciplinary process that requires a whole team of experts. This paper points out two groups of data that we find most relevant.

The first group refers to data on the distribution of poisons and products containing poisonous agents on Croatian territory (location of its production, its storage, transportation, application, import, export and uncontrolled disposal). As a very complex and comprehensive task, it requires wide cooperation of all production subjects and some government institutions. We have prepared and presented in this paper a proposal of a form to be used for collecting data in the field and a proposal of a respective database.

The second group contains data on poisons (physical and chemical properties, half-life, ground, water and air behavior, toxicity for living organisms, antidotes, treatment, emergency response...). The experience of other countries and our own shows that the best way in collecting such data is to utilize the existing databases. Based on our work experience with two toxicological databases: IRTPC and TOXLINE, the paper briefly outlines the advantages of such a great amount of data available at any moment.

*Key words:* accidents, toxicology, data collecting, database

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## POTABLE WATER SUPPLY ON THE LIBERATED RIGHT BANK OF THE RIVER KUPA

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Water supply in the period following the combined police and military operation "Storm '95" was monitored in six villages and eleven hamlets/settlements on the right bank of the River Kupa. The houses in the area were destroyed and burnt down. Since 36 of 47 household wells were not accessible for testing, water samples were collected from the remaining 11 wells. Sample analyses showed that the water from the wells was not potable due to pollution by heavy metals, organic matter and motor oil. Consequently, potable water had to be supplied by tank trailers from other regions of Croatia.

*Key words:* military operation, heavy metals, potable water

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## THE EFFICIENT REDUCTION PROCEDURE OF THE ORGANOPHOSPHORUS INSECTICIDES CONTENT IN THE SOLUTIONS

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**T**he water supply was an essential issue during the siege of Sarajevo. The system for the purification of water from open courses of the rivers Miljacka and Moščanica was installed by financial support of the Soros foundation. The major part of the town was supplied by this water, commonly termed as technical. The regular investigations of water samples from these installations during the first six months have indicated a presence of certain insecticides.

However, Sarajevo citizens used the water for drinking and cooking since the heavy shelling often hindered movement to the drinking water supply points. The situation urged us to find a simple and efficient method to reduce the content of these toxic substances in the water.

Solutions of different concentrations of organophosphorus insecticides were passed through a mixture of solid aliphatic hydrocarbons and later identified by chromatography and spectrophotometry.

As a result, we managed to reduce the insecticides content for 30-50% with respect to the standard solutions.

*Key words:* organophosphorus insecticides, water, reduction

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## MODIFIED DETECTION OF IRRITANTS AND VESICANT GASES

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**I**n the situation of total siege, the inhabitants of Sarajevo were exposed to frequent shelling with chemically charged missiles. The means of identifying poison gases were predominantly in the hands of former JNA (Yugoslav People's Army), while the ones in the hands of Civil Defense were often obsolete and the movement in the city was hardly possible. Without fuel, water, chemicals and other equipment, unable to use measuring instruments, it was necessary to find a quick and reliable way to identify chemical agents, and consequently adjust preventive and protective measures for the population. It was possible to renew some of the indicator vials for the poison gas detection, but the only alternative left was to create new approaches to identification of toxic matter.

*Key words:* aquatic environment, irritant, vesicant gases

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## ENVIRONMENTAL HEALTH AND MONITORING IN ESTONIA

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The struggles of a nation during and after a conflict with other nations include efforts to maintain a healthy environment for the population. An assessment of environmental health and monitoring in Estonia after the dissolution of the Soviet Union has shown that the country suffered from neglect during the "cold war" after World War II, and efforts to improve the status quo have been slow since independence was gained in 1991. The delay in improvement of environmental health and monitoring can be attributed to many factors, such as low political will; other government priorities; lack of funds, public attitudes; and resistance to change. International assistance programs as well as efforts by Estonians have led to some change and progress in environmental management since 1991. An examination of the recent history of this small country provides examples of environmental neglect and consequences, as well as recommended corrective measures. Examples of environmental pollution which can be associated with adverse effects on human health and the environment will be provided. The origin and consequences of pollution by radioactive materials, emissions from very large power, central heating, and cement plants, activities at military bases and installations and at air fields, shale oil production, and vehicle emissions, among other sources, will be discussed.

*Key words:* environment, health, pollution, progress, Estonia

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## PROBLEM OF ELECTRIC- POWER FACILITIES DESTROYED DURING THE WAR IN CROATIA

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When the war destruction of electric power facilities in Croatia was first reported, the scientific community was alarmed at the prospect of leakage of PCB cooling oils into the environment, particularly in the karst. Some reports predicted catastrophic consequences for the environment. In 1992, the Toxicological Office of the Croatian Ministry of Health started to collect data on destroyed electric power facilities cooled by PCB oils. The data showed, however, that Croatia's PCB oil disposal difficulties were far less significant than those of the Western Europe, thanks to a limited number of facilities using PCBs. Most Croatian electric-power transformers used non-PCB oils. According to data collected from the national agency for electric distribution, electric-power condensers were using approximately 500 t of PCB oils. Non-PCB oils from destroyed transformers in the amount of 300 t were spilled or burned. About one tone of PCB oils from destroyed condensers leaked into the environment or burnt on the whole territory of the Republic of Croatia. By now, not a single study investigated the environmental contamination with PCB oils as a war consequence in Croatia.

*Key words:* electric-power facilities, PCB, war

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## Pb AND Cd IN VEGETABLES CULTIVATED IN A WAR REGION

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**D**ifferent anthropogenic sources of pollution (industry, traffic, mineral fertilizers, plant protection agents, etc.) add to the already present quantity of heavy metals in soil from geogenic sources on a daily basis. Each ammunition explosion in the war-ridden Croatia contributed to that quantity. The accumulation of heavy metals in plants correlates partly to its concentration in soil. We therefore took interest in determining whether four years of intensive war destruction (many Croatian regions were bombarded and shelled by tons of ammunition) affected vegetables cultivated in the war regions as to augment concentrations of heavy metals. Pb and Cd were determined in white cabbage and potato cultivated in a war region (Eastern Slavonia) and compared with samples collected from regions not affected by the war. Pb and Cd were determined by voltametric analysis.

*Key words:* heavy metals, Pb and Cd, vegetables, war region

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## NITRATE AND NITRITE IN VEGETABLES CULTIVATED IN WAR REGION

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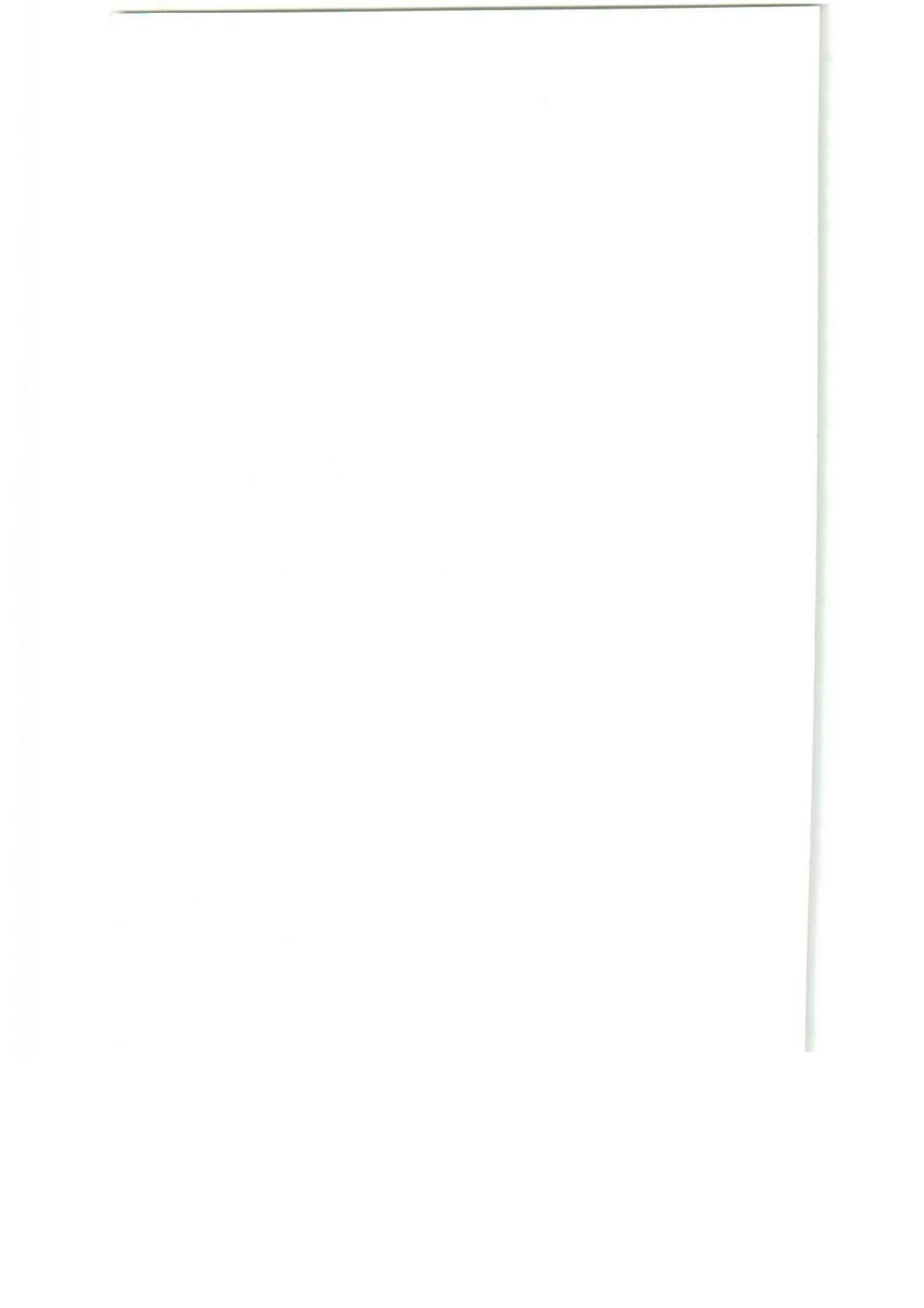
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**I**n addition to nitrates present in soil, water and plants as a consequence of nitrogen fixation and due to a wide use of nitrogen-based fertilizers in the agriculture, different compounds from explosives and propellants can release surplus  $\text{NO}_3^-$  and  $\text{NO}_2^-$  to soil, water and consequently to plants as a result of biological and chemical degradation in the environment. To determine whether four years of war destruction affected  $\text{NO}_3^-$  and  $\text{NO}_2^-$  content in vegetables, we examined white cabbage and potato cultivated in a war region (Eastern Slavonia) and compared with samples from a region not affected by the war.  $\text{NO}_2^-$  was determined by spectrophotometry and  $\text{NO}_3^-$  by ion-chromatography of the same extract.  $\text{NO}_3^-$  content ranged from 14 to 358 mg/kg of fresh potato and from 0.08 to 0.32 mg/kg of fresh cabbage, while  $\text{NO}_2^-$  content ranged from 0.19 to 0.52 mg/kg of fresh potato and from 0.08 to 0.32 mg/kg of fresh cabbage. The content of  $\text{NO}_3^-$  and  $\text{NO}_2^-$  did not significantly differ from the control.

*Key words:* nitrate, nitrite, vegetables, war region

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SECTION 7

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## Free Communications





## REVIEW OF THE TOXICOLOGICAL SCREENING RESULTS (TOXI-LAB) FOR YEARS 1985-1994

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**T**oxicological analysis uses screening tests whenever accidental or intended medication poisoning, or drug abuse, are suspected. Diagrams show the results of the toxicological analyses for years 1985-1994. Drugs (opiates, cannabinoides, cocaine) and medicaments (phenothiazines, benzodiazepines, tricyclic antidepressants) are determined quantitatively or semiquantitatively after the thin-layer chromatography screening of the biological material. The increase in positive findings for most substances can be observed for the year 1990, as well as for the years 1992-1994. The increase in opiate findings is due to addict control program carried out by "Sestre milosrdnice" Clinical Hospital. The years 1992-1994 marked a notable increase of samples containing benzodiazepines. Despite the increasing number of newly available substances of abuse, data for those years show a decrease in the number of undetermined samples.

*Key words:* screening tests, medicaments, drugs

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## PROTECTION FROM TOXIC EFFECTS IN PRODUCTION OF HI-6, AN ORGANOPHOSPHORUS COMPOUND ANTIDOTE

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**W**e have presented a model of a "closed system" for synthesis of HI-6, acetylcholinesterase reactivating agent. In the first step of synthesis, the reactant is 1,1'-di(chloromethyl)ether, a carcinogenic toxicant.

A classic protection in semi-industrial synthesis is presented through the effects on working environment. The introduction of specifically assembled equipment, inert atmosphere and semi-automatic procedure performance, excludes any loss of dichlorodimethylether from the system.

*Key words:* oxymes, pesticides, 1,1'-di(chloromethyl)ether

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## CHROMIUM, NICKEL AND MANGANESE IN STAINLESS STEEL COOKING UTENSILS AND PERTINENT REGULATIONS

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**A**ssaying chromium nickel and manganese for specific migrations with a 4%-acetic acid under specific time and temperature conditions is an obligatory part of hygienic safety surveillance.

Routine control of a large number of imported cooking utensils has revealed a specific fluctuation of values above the limit for chromium and/or nickel, and less frequently so for manganese. After a repeated (second) extraction, further tests showed that the levels of all three metals were within limits for the majority of samples previously found unfit.

These findings considered as well as the longer period of use, the introduction of amendments to the current regulations should appear justified.

*Key words:* hygienic safety, stainless steel, regulations

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## THE NEED FOR ALTERNATIVES IN TOXICOLOGY TESTING

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A variety of research methods, including non-animal systems, laboratory animals, and clinical testing with human volunteers, is used in product safety evaluation to gain the knowledge needed to protect human health and safety. These data must be generated to insure that products can be used safely and under reasonably foreseen abuse situations. In addition, such information is required to provide safe and healthy work environments. All types of testing interrelate like pieces of a puzzle and may provide important information toward developing a more complete picture of the safety of a new ingredient or product. Non-whole animal methods continue to be developed in hope of replacing animal tests whenever possible. Furthermore, such tests can offer insight into the mechanistic puzzles underlying animal toxicity and pathophysiology. However, because of the complexity of the biological system and the lack of an understanding of mechanisms of toxicity, current alternatives are limited. The pursuit of valid, scientifically sound alternatives must continue to be the goal of the scientific community. Because of the need for animal testing, work continues in developing improved protocols that reduce the number of animals needed for a particular study and that minimizes or eliminates discomfort for test animals. Methods that replace, reduce, or refine animal use are known as alternatives. A review of alternative research in safety evaluation with particular emphasis on the cosmetic industry will be examined.

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## SKIN AS AN EXPOSURE MEDIUM

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**D**ermal exposure to chemicals occurs in both domestic and occupational environment and may lead to systemic effects. Before reaching the systemic circulation, a chemical has to cross several potential barriers. Stratum corneum, the outermost layer has been identified as the principal barrier for penetration of most chemicals. Stratum corneum is seen as a wall-like structure with protein bricks and lipid mortar, creating hydrophilic and hydrophobic "channels". *In vivo* percutaneous absorption is a complex biological process as there are many variables involved that are often strongly interrelated. Beside the structure of the stratum corneum through which it has to permeate, the physical and chemical properties of the penetrant, such as differential solubility in water and fat, polarity and molecular size, play an important role in determining its percutaneous absorption. The prediction of skin penetration is extremely complex, being influenced by a number of environmental factors such as temperature and humidity as well as by skin dependent variables such as skin condition and anatomical site. In studying dermal absorption, volunteer studies should be favored to *in vitro* and *in vivo* animal studies, since they provide definitive data for human risk assessment of dermal exposure to chemicals.

*Key words:* dermal exposure, skin penetration, environmental factors, volunteer studies

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## HYDROLYTIC ENZYMES ACTIVITY IN THE LIVER OF THE CARP (*Cyprinus Carpio L.*) TREATED WITH LEAD, MAGNESIUM AND SELENIUM

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**E**lement accumulation in the cells reflected on the lysosomal population and can be observed by investigating the lysosomal enzymes activity. We observed the acid phosphatase activity in the liver of the carp (*Cyprinus carpio L.*) treated for 80 days with constant concentrations of 0.3 mg/L Pb ( $Pb(NO_3)_2$ ), 0.1 mg/L Mg ( $MgCl_2$ ) and 0.1 mg/L Se ( $Na_2SeO_3$ ), and sampled each 7 days. Histochemical analysis indicated that stronger acid phosphatase activity was caused by the lysosome growth. Changes were characteristic for each of the three investigated elements. Lead caused formation of many small peribiliary located lysosomes. During the magnesium treatment lysosomes were heterogeneous, their size varying from small to large ones, distinctly peribiliary located. The most prominent changes occurred during selenium treatment; lysosomes were large and numerous. The increase in the lysosomal number caused by Pb, Mg and Se was most significant during the first two weeks of treatment. Subsequent changes in the lysosomal number and size were less expressed but the acid phosphatase was enhancing. After 60 days, we observed diffuse activity of the acid phosphatase indicating damage of lysosomal membranes. After 80 days, necrotic changes in the liver tissue started to show. Beside the lysosomal population, the investigated elements caused changes in the blood sinusoid walls. The investigation of alkaline phosphatase showed intensified activity in the perilobular sinusoid walls, which indicated changes of transmembranal transport.

*Key words:* liver, hydrolytic enzymes, lead, magnesium, selenium

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## THE EFFECT OF OCHRATOXIN A ON CONCENTRATIONS OF IRON AND ZINC IN RATS

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The recent years saw an increased interest in ochratoxin A (OA) due to its possible role in the aetiology of Balkan endemic nephropathy (BEN). Anaemia is one of the earliest symptoms of BEN, and the disturbance in protein synthesis is a possible toxic effect of OA. The aim of this study was to evaluate the effects of OA on concentrations of iron and zinc, two essential elements in hematopoiesis and protein synthesis. The experiment was performed on adult female Wistar rats. The animals were administered 0 (control), 500 or 1,000 µg OA/kg b.w. ip. every second day over a four-week period. At the end of the exposure, the elements were analysed in tissues and in urine by electrothermal atomic absorption spectrometry. The exposed animals manifested significantly lower body weight and fresh weights of the liver, kidney and femur than controls. No effects were found either on tissue iron concentrations or blood parameters. Zinc concentrations in rats treated with the higher dose decreased in the liver and kidney, and increased in the urine. We concluded that the rat exposure to OA interfered with concentrations of zinc as an integral part of DNA-polymerase and necessary for DNA and RNA synthesis. Further investigations are needed to clarify whether OA affects zinc *in vivo* directly, or through some other mechanism(s) causing the decrease in zinc tissue concentrations.

*Key words:* ochratoxin A, hematopoiesis, iron, zinc, protein synthesis, rat

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## ARE DMSA ITS MONOISOAMYL ESTER TRANSFERRED THROUGH MAMMARY GLAND IN RATS?

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**M**onoisoamyl ester (Mi-ADMS) of dimercaptosuccinic acid (DMSA) has been found to be a very efficient antidote in removing inorganic mercury from the body of lactating dams and their pups. When chelation therapy was given to lactating dams after <sup>203</sup>Hg IP administration, both dams and pups showed reduction of mercury body stores. Mi-ADMS was more efficient than DMSA in this respect. However, it was not clear whether the chelator primarily bound metal in dams or it also passed through the mammary gland in its original form to mobilize mercury in pups. The answer to this question was looked for in this experiment.

The same day we gave radioactive mercury (<sup>203</sup>Hg(NO<sub>3</sub>)<sub>2</sub>) to 8-day-old sucklings, DMSA or Mi-ADMS were administered to lactating dams (0.5 mmol/kg for 5 days). The whole body radioactivity of pups was measured daily for 7 days. We found that at the end of the experiment whole body retention of <sup>203</sup>Hg in 15-day-old sucklings did not differ significantly from the control group, despite the treatment of lactating dams with chelators. Therefore, it was evident that neither Mi-ADMS nor DMSA can be transferred through the mammary gland in their original form and act as chelators for mercury in pups.

*Key words:* monoisoamyl ester, DMSA, mercury-203, lactation, rats

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## ACTIVITY OF THE T-2 MYCOTOXIN ON THE SERUM PROTEINS DURING IMMUNIZATION OF CHICKS

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**T**he control group A of chicks did not receive food mixed with mycotoxins. The experimental group B was given food containing T-2 toxin (0.5 mg/kg) during the second and the third week of their life and the group C during the fourth and fifth week. The group D was given food containing T-2 toxin during the whole fattening period. All 14-days-old chicks were administered New Castle disease vaccine.

Prealbumin almost doubled during the T-2 toxin treatment of four and five weeks old chicks. Albumin, alpha globulin and the total protein quantity in the serum did not significantly differ between the groups. Group D manifested a significantly lower production of gamma globulin in serum (6.0 g/L) than in the other groups. The fact suggests that a long term T-2 toxin exposure causes immunosuppression.

*Key words:* T-2 mycotoxin, serum proteins, immunosuppression

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## BRONCHOALVEOLAR LAVAGE IN THE INVESTIGATION OF RESPIRATORY DISEASES - A METHODOLOGICAL APPROACH

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**T**he study of the respiratory pathophysiologic mechanisms, their origin and development, the toxicokinetics of agents in the lung compartments, and the interaction of endogenous cell and tissue with exogenous pollutants still present a big challenge for the scientific community. Our examination of an animal model aimed at gaining an insight into the particle and fiber alveolar clearance and distribution, as well as into their translocation and retention in the lung and lymph node tissue. The lungs of 120 Wistar rats were examined after application of spherical and fibrous inorganic agents. We applied two useful laboratory methods that may provide promising results in cytotoxicology: intrabronchial instillation of agents into the rat lungs and bronchoalveolar lavage of the rat lungs.

The intrabronchial instillation method enables good control of the applied agent quantity and of its concentration before and after application. Bronchoalveolar lavage of the lungs enables access to the lung free cell population and to other soluble constituents of the lung microenvironment. The described methods allow insight into the series of events in the lung compartments after initial deposition of agents.

*Key words:* intrabronchial instillation, bronchoalveolar lavage

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