

Session 2: Toxicology

[S 024] The Effects of Four Weeks Treatment with Different Doses of the Pesticide Dimethoate and/or Arsenic on the Chromosomes of Young Rats

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The aim of the study was to investigate in animal model experiments, analyzing sensitive genotoxicity endpoints as effect-biomarkers, whether the toxic effect of the often used organophosphate pesticide dimethoate would be influenced by contamination of arsenic. This problem had to be investigated because in several big agricultural regions of Hungary higher arsenic content than considered safe was found in the drinking waters. Treatments were made by the two compounds alone and by administering them together respectively. Outbred Wistar rats, 10 per groups, starting at their age of 30 days were treated by gavage for 4 weeks, 5 days per week. Doses applied were: two dimethoate doses 7.0 mg/kg (1/100 LD₅₀) and 9.33 mg/kg (1/75 LD₅₀), two Natriumarsenit doses: 6.4 mg/kg (containing 3.38 mg/kg As) and 26.61 mg/kg (containing 15.3 mg/kg As), further the lower dimethoate doses with the low and high Na-arsenit doses respectively, and the higher dimethoate doses similarly with the low and high Na-arsenit doses respectively were administered. There was an untreated control group and one treated with distilled water (altogether 10 groups were examined). After finishing treatments the chromosomes from the bone marrow of the femurs were prepared and 20-20 metaphases were evaluated in each animal. There have been estimated the numerical and the structural chromosome aberrations, as well as the changes in body

and organ masses. Statistical evaluation was done by ANOVA, post hoc analysis by LSD probe, the changes of the chromosomes by Fisher's test. Dimethoate in itself did not influence the general toxicological parameters. The Na-arsenit doses induced changes in the organ weights. This was still altered by the mutual administration. The higher dose of dimethoate in itself enhanced the number of numeric aberrations by causing the appearance of aneuploid cells. Combination with As gave the same result. The compounds in themselves alone did not cause the emergence of more structural aberrations than there were in the controls. The effects on the structural chromosome aberrations of both doses of dimethoate were enhanced by the smaller dose of Na-arsenit, mainly the number of breaks and acentric fragments had grown significantly. The higher dose of Na-arsenit, probably because producing already overall toxic effects, obstructed the fine changes in the chromosomes. The results point to the fact that dimethoate and arsenic seem to potentiate each others effect. Thus at the areas of Hungary where water As-content were high, standards for safe dimethoate level might be overridden and the two compound together might cause early damages in the sensitive parameters of the mammalian body, which should be taken into consideration when establishing safety borders.

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[S 025] Comparison of Genotyping and Phenotyping for Routine Diagnosis of GSTT1

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Purpose: The sensitivity, specificity, and reproducibility of a PCR-based genotyping method for routine diagnosis of glutathione S-transferase (GSTT1) in patient samples was evaluated and compared to our in-house phenotyping assay.

Methods: Blood from 1777 patients was tested in parallel with both methods. For genotyping, DNA was isolated from 200 µl blood with the QIAGEN Tissue Kit, amplified in multiplex PCR with primers for GSTT1, GSTM1, and β-globin as internal control (modification of method described by ARANDT et al. 1996), and detected in 2.5% agarose gel electrophoresis. Conjugators (480 bp GSTT1 band present) can be clearly distinguished from nonconjugators (GSTT1 band absent). For phenotyping, 3.5 ml blood was incubated with

methyl bromide (MeBr) at 37°C for 60 mins and the decrease of MeBr determined by gas chromatography. Conjugators show a decrease of MeBr of > 70% while nonconjugators show a decrease < 60%. Values between 60 and 70% are indeterminant.

Results: All 177 samples could be genotyped, with 27% showing the GSTT1 nonconjugator status (literature: ca. 25%); 55% showed the GSTM1 null genotype (literature: ca. 50%). Reproducibility as tested on 30 samples was 100%. Of the 157 samples (89%) yielding phenotypic results (11% were in the indeterminant range), 22% were nonconjugators. The correlation between genotyping and phenotyping was 99% (156/157), with the one exception

reproducibly negative in PCR but phenotypically positive (74%). Compared to phenotyping, PCR showed a sensitivity of 99% and a specificity of 100%.

Conclusion: The genotyping method described here shows a high sensitivity, specificity, and reproducibility compared

to our in-house phenotyping assay. In addition, it allows detection of all samples (phenotyping yielded 11% indeterminate results), requires less blood, and permits simultaneous detection of GSTT1 and GSTM1, further reducing costs and labour. The method is, therefore, recommended for routine diagnostic laboratories.

[S 026] Chlorophenol Excretion – Results of Biomonitoring for a Reference Group and for Harbour Mud Workers

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Objectives: Assessment of reference values for 13 di-, tri-, tetra- and pentachlorophenols (Di-, Tri-, Tetra- and PCP) in urine for a group of adult Germans and evaluation of the corresponding biomonitoring data for harbour mud exposed persons.

Methods: 96 men (aged 47 ± 10 y) of northern Germany working in administrative jobs and 83 workers (aged 46 ± 11 y) exposed to harbour mud during deepening the fairway of the river Elbe were investigated in 1996/97. Anamnestic data (e.g. life style factors as smoking habits, diet) were

additional CPs (2,6-DiCP; 2,3,6-TriCP; 2,3,4,5-TetraCP) were excreted in only few persons of both groups in small amounts. For 6 CPs, median and 95percentiles for reference and harbour group are as follows:

For 2,5-DiCP, 2,4,6-TriCP and PCP, the Mann-Whitney U test was applied and indicated significantly higher excretions for the harbour workers ($p < 0.05-0.01$) vs. the reference group (with or without creatinine adjustment). The individual harbour workers' CP excretion, however, ranged within the environmentally caused concentration ranges given in

	Reference group n = 96					Harbour workers n = 83				
	>d.l. %	median		95perc.		>d.l. %	median		95perc.	
		µg/l	µg/g	µg/l	µg/g		µg/l	µg/g	µg/l	µg/g
2,5-DiCP	63.5	0.54	0.43	6.2	5.2	89.2	1.0	0.64	39.1	20.4
2,4/3,4-DiCP	36.5	<0.20	<0.14	1.5	1.1	56.7	0.30	0.14	2.5	1.2
2,4,6-TriCP	62.5	0.37	0.24	1.8	1.3	75.9	0.50	0.33	1.9	1.8
2,4,5/2,3,5-TriCP	22.9	<0.30	<0.22	0.6	0.3	22.9	<0.30	<0.22	0.7	0.6
2,3,4,6/2,3,5,6-TetraCP	31.2	<0.50	<0.36	1.3	1.0	32.5	<0.50	<0.36	2.1	1.5
PCP	80.2	1.2	0.90	5.3	3.6	88.0	1.6	1.3	11.5	6.8

collected by a standardised questionnaire. Urinary excretion of 13 chlorophenols was analysed with a sensitive and reliable gas chromatography/mass spectrometry method after hydrolysis, clean-up by steam distillation, extraction steps, enrichment and derivatisation (limits of detection (d.l.): 0.2-0.5 µg/l). Creatinine was measured by the enzymatic/photometric procedure.

Results: 4 CPs (3,5-; 2,3-DiCP; 3,4,5-; 2,3,4-TriCP) were not found in any urinary specimen of both groups. Three

the literature. Age, smoking habits and fish consumption showed no significant influence on CP excretion in both groups.

Conclusions: 95percentiles for the reference group are lower for most Di- to TetraCPs compared to the rare literature data, especially for 2,5-DiCP in USA. Three CPs, among them PCP, showed small, but significant increases in the harbour group. The workers' overall CP excretion, however, does not exceed the environmentally caused concentration ranges.

[S 027] Cosmetics in the Ecosphere: Synthetic Musks and UV-Screens

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Pollutants in human milk play an important role in the estimate of total chemical load in humans. Human milk contamination not only functions as an important bioindicator of human fetal load but in addition demonstrates regional patterns in environmental pollution. The search for additional persistent new contaminants in human milk samples eventually led to the discovery of persistent cosmetic chemicals like synthetic musks and UV-screens in human milk and in fish and mussels.

Nitro musks and polycyclic musks are in use as substitutes for natural musks derived from the musk deer (*Moschus moschiferus*). Toxicity data on synthetic musks, especially on polycyclic compounds are still scarce. In our long term developmental study on musk xylene (MX), we found that two week old offspring of chronic musk xylene exposed male and female Long Evans rats (MX: 0.07, 0.7, 2.0, 7.0 or 70mg/kg/d) exhibited dose dependent MX concentrations in their body fat and a corresponding induction of Cyp 1A1 and 1A2 in liver microsomes. Chronic exposure to the highest dose of MX led to induction of Cyp2B in both parent and offspring generation, whereas Cyp3A induction was solely detected in offspring. Cyp1A1 and Cyp 1A2 was found to be moderately induced at birth indicating transplacental induction. In addition, parent and offspring exhibited increased HDL-cholesterol levels following the highest dose

of MX. At that dose also some developmental and reproductive toxicity was observed.

UV screens are organic chemicals absorbing UV radiation of specific wavelengths and emitting the absorbed energy as fluorescent light or heat. UV-screens are used to protect the skin from intense UV radiation (ozone whole) and are important in the view of increasing numbers of skin cancer. UV-screens are increasingly added to other cosmetics like bubble baths, hairsprays, lipsticks, beauty creams, and even to children's clothing. Sunscreens, present in water below the detection limits, have been found to bioaccumulate in fish. In Germany, six out of seven UV-screens are detected in fish. On the basis of structural considerations, we investigated possible estrogenic effects of several UV screens. The three compounds MBC (3-(4-Methyl-benzylidene)-camphor, OMC (Octyl-methyl-p-aminobenzoic acid) and BP-3 (Benzo-phenone-3) induced cell proliferation in the in vitro E Screen test using transformed human breast cancer cells (MCF-7 cells), while other chemicals used as UV-screens were less active. The chemicals promoting cell proliferation also induced the expression of the estrogen dependent protein pS2 by MCF-7 cells. According to preliminary results from the in vivo identification assay (uterotrophic assay), the UV-screens found active in vitro also appear to promote increased uterine weights.

[S 028] Is Dioxin Poisoning Still a Matter of Concern?

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The Seveso accident led to world wide search for other sources of PCDD/Fs and their reduction. The "Seveso Directive" for control of major accident hazards involving dangerous substances was implemented in member states of the EU. Austria was a trendsetter in lowering the emission standards for waste incinerators. From the remaining emissions almost 50% can be attributed to domestic heating and 25% to metal industry. The annual decrease of PCDD/Fs in human milk was around 10% in Austria in the 1990s. Occupational exposures, however, are still a matter of concern, demonstrated by the recent poisoning of 2 young female office workers of a textile research institute in Vienna.

Both experienced a worsening of their symptoms after the attempt to cleanse body and skin by losing weight. In a follow up of male cases with chloracne since 1969 - 1975 we observed an increase of TCDD in blood even in the late phase of poisoning if they reduced weight. Therefore we

discourage attempts to mobilize dioxins from body fat by fasting. We recommend life-long preventive check-ups including liver function tests, because in a case-control study with 50 cases of chloracne and 100 unexposed controls we found persistent signs and symptoms of disease related to plasma levels of TCDD even 25 years after begin of exposure. The blood lipid concentrations at last check-up of this cohort ranged from 19 to 2900 pg/g. Men with a history of liver disease had higher levels (801 pg/g) than without (407 pg/g). Multiple regression analysis with the factors age, alcohol consumption, BMI and log TCDD showed significant effects of TCDD and its interaction with age, indicating higher liver transaminases after high TCDD at young age. 48% of exposed were found to have an abnormal coproporphyrin isomer ratio related to plasma TCDD-level. These results contribute to the evidence that chloracne is not the only chronic disease which can be related to TCDD

exposure, even 23 years after main exposure ended. The possible increase of latency with decrease of exposure level suggests to look for latent effects in children of exposed mothers even after environmental exposures.

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[S 029] Lead Biomonitoring Study in Arnoldstein/Kärnten

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An important industrial complex is situated in Arnoldstein/Kärnten (Carinthia). Due to a lead smelter there was a long-time exposure with lead in this area. In June 1992 the population at risk was screened for lead levels in blood and teeth. Mandated by the Government of Carinthia, the Institute of Forensic Medicine (G.D.) reported of increased lead levels in humans. Mainly the blood lead levels of children were found to be intolerably high. This report resulted in the sanitation of the area. In 1998 the Government of Carinthia (O.F.) wanted to obtain an expert opinion (G.D.) on the results of the sanitation. Concentrating on children, the population most at risk, a biomonitoring study was carried out in June 1998. 284 volunteers were examined including questionnaire (n = 284), blood sample (n = 250) and teeth collection (n = 59) (S.B.) and neuropsychological testing (n = 76) (U.O.). Blood lead levels were analysed in 250 samples (G.R., G.D.). The results are shown in the **table** below.

A significant decrease in blood lead levels was found from 1992 to 1998. As well a decrease of all intra-individual blood lead levels was found from 1992 to 1998. The "Center for Disease Control" (CDC) and the "German Human-Biomo-

onitoring" recommends blood lead levels < 10 mg/dl. In 1998 all blood lead levels in Arnoldstein were < 10 mg/dl. In 1992 and in 1998 a significant trend of lower blood lead levels was found in relation to the increasing distance of the home to the lead smelter.

In September 1998 the study population received a letter with their individual results. The next day the concerned population of Arnoldstein, the local and regional politicians and the media were informed. This is in our opinion a reasonable model of proper risk communication of environmental health studies.

59 dentine lead levels were analysed (G.R., G.D.). Dentine lead levels decreased from 1992 to 1998. 76 children and teenagers were neuropsychologically tested (U.O.) with different test batteries (CF-20, ZVT, LPS 13+14, LPS-13, ZN). The association between lead levels and IQ was tested by multivariate regression analysis adjusting for potential confounders (G.S., A.S., U.S.). Possible correlations of IQ and specifically the capacity for concentration and lead levels will be presented and discussed at the conference.

		Blood lead level 1992 µg/dl	Blood lead level 1998 µg/dl
Children 0-9 years	Median	7.48	3.43
	Average	9.14	3.67
	Maximum	38.90	8.50
	Number	238	145
Youth 10-19 years	Median	5.95	2.82
	Average	6.72	3.17
	Maximum	25.50	9.95
	Number	182	105