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to air and soil pollution. A thorough epidemiological survey was conducted and showed a moderately elevated blood lead concentration (40 $\mu g\ L^{-1}$) as a result of local exposure.

MATERIAL AND METHODS

Households with a soil lead concentration >100 mg $kg^{-1}$ were selected (local normal value: 65 mg $kg^{-1}$). Dog owners were identified by the veterinary clinics of the area. Animals were selected if they were >3-month old and residing for >2 month in the area. A questionnaire was used to determine the potential presence of lead sources in the household and exclude these dogs from the study. Breed and gender were recorded. Exposure parameters such as indoor or outdoor residence, distance from the smelter were used for statistical analysis. A control group was selected in a residential area with no known lead exposure. For each dog, a blood sample was drawn (5 mL), identified and analyzed by atomic absorption spectrophotometry. Nonparametric tests (Mann–Whitney and Kruskall–Wallis) were used to compare lead values. A *P*-value of 0.05 was considered significant.

RESULTS AND DISCUSSION

Forty-eight dogs were eventually included in the survey (22 in the study area, 26 in the control area). There was no significant difference between groups as far as age, gender and time spent outside were considered. Median blood lead concentration was <40 $\mu g\ L^{-1}$ in the control animals and 68.4 $\mu g\ L^{-1}$ in the exposed animals. The populations appeared statistically different. For animals living mostly outside, median blood lead concentration was 86.4 $\mu g\ L^{-1}$, significantly higher than the median value for dogs living inside (58.2 $\mu g\ L^{-1}$). Our study confirmed that dogs living near the smelter had a higher blood lead concentration, even higher than children did in the same area. These values are not considered toxic in dogs [2]. Values measured in urban (control) dogs are quite low. Other studies conducted in dogs living in urban areas showed that they are declining [3], thanks to the suppression of leaded gasoline. It would be interesting to re-investigate in a larger sample 'normal' blood lead concentrations in urban dogs.

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## G-19 (0182)
## Isoproturon – a partial uncompetitive inhibitor of microsomal glutathione *S*-transferase of Wistar rat testis

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INTRODUCTION

Isoproturon [3-(4-isopropylphenyl)-1,1-dimethylurea], is a herbicide widely used in Portuguese agriculture for control of annual grasses and broad-leaved weeds in winter cereals. The persistence of isoproturon in soils and aquifers could be a source of animal contamination by this pollutant. Several authors observed a significant *in vivo* genotoxic effects such as chromosome aberration and sperm-shape abnormality in Swiss albino mices exposed to a dose of 200 mg $kg^{-1}$ [1]. Unfortunately, scarce information is available regarding metabolic effects of isoproturon on testis detoxication metabolism. The aim of this work was to study the *in vitro* effects of isoproturon on microsomal glutathione *S*-transferase (mGST), determining its influence on testis conjugation reactions.

MATERIALS AND METHODS

The experiments were performed with Wistar rats (200 g) from the *Animalarium* of LTB. The animals were housed in a controlled temperature room (25 °C) on a 12 h light-dark cycle and allowed free access to rat chow and tap water *ad libitum*. The animals were killed by decapitation and testis are immediately removed and perfused with ice cold 0.154 M KCl 50 mM Tris-HCl, 7.4. Tissue was homogenized with ice cold 0.154 M KCl 50 mM Tris-HCl, pH 7.4. at 5500 rpm, six impulses, with homogeneizer Potter type. Homogenates were centrifuged at 10 000 *g* for 20 min, at 2–4 °C. Microsomes were precipitated by centrifuging the postmitochondrial supernatants at 105 000 *g* for 60 min [2]. Microsomes were used to estimate mGST activity for 1-chloro-2,4-dinitrobenzene (CDNB) according to Habig *et al.* [3], using a concentration range of isoproturon from 5 to 75 μM in assay mixture. The kinetic studies were performed using a substrate concentration range from 15 to 500 μM. Protein concentration was determined by the method of Lowry [4]. Data were expressed as mean ± SD of five replicates. Significance was calculated using ANOVA-one way and Duncan test ($P < 0.01$) [5].

RESULTS

Our results showed that isoproturon induced a concentration-dependent decrease of mGST activity for a range of concentration from 5 to 75 μM, appointing isoproturon as an inhibitor of mGST. The kinetic studies confirmed the above hypothesis and

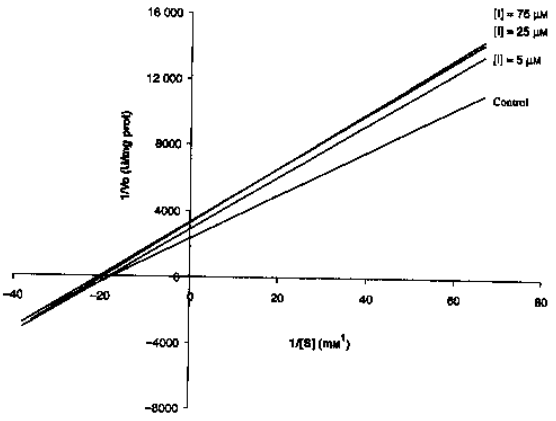


**Fig. 1.** The 1/v0 vs. 1/[S] plot in the presence of different fixed concentrations of isoproturon, as a partial uncompetitive inhibitor ($0 < \alpha < 1$, $0 < \beta < 1$) of mGST.

suggest isoproturon as an *in vitro* partial uncompetitive inhibitor ($K_{iu} = 0.5$) of testis mGST.

DISCUSSION

In the current work we study the *in vitro* effects of isoproturon on testis mGST and we try to determine its inhibition mechanism. The obtained results showed that isoproturon inhibits this enzymatic activity by a partial uncompetitive mechanism. Considering that mGST plays a protective role as a key enzyme of detoxification pathways, we suppose that this metabolic interference on the testis conjugation reactions can eventually affect spermatogenesis, disturbing fertility of male Wistar rats.

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## G-20 (0216)

## Immunochemical evaluation of plasma vitellogenin in the sea bass (*Dicentrarchus labrax*, L.) following exposure to 17β-oestradiol and 4-nonylphenol

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INTRODUCTION

Alkyl phenols, such as 4-nonylphenol (NP), widely used as plastic additives and as surfactants in detergents, are widely released in the environment from different sources. These chemicals are able to induce detrimental effects on endocrine, reproductive, and immune systems in several animal species, including fish. NP have been shown to induce oestrogenic effects as indicated by the induction of plasmatic vitellogenin (Vtg) in different fish species [1,2]. The induction of plasmatic Vtg is considered a biomarker for the oestrogenic effect in immature or male fish, as Vtg is physiologically only synthesized in mature female fish. The aim of this study was to evaluate by immunoenzymatic assays with homologous anti-Vtg antibodies the effects of 17β-oestradiol (E2) and NP on Vtg induction in the sea bass (*Dicentrarchus labrax*, L.), an euryaline species intensively farmed in the Mediterranean basin.

MATERIALS AND METHODS

Immature (20 ± 3 g) sea bass were injected i.p. with NP in corn oil at the dose of 25mg $kg^{-1}$ or 50 mg $kg^{-1}$, given in half doses 1 week apart. Blood samples were collected on day 14 after the beginning of treatment. One group treated with a single dose (5 mg $kg^{-1}$) E2 acted as positive control. Another group treated with corn oil acted as negative control. The degree of Vtg cross-reaction of plasma samples was assayed by Western blotting. Plasma levels of Vtg were determined by an indirect ELISA assay [2,3] using an homologous sea bass polyclonal Vtg antibody. As anti-sea bass Vtg antibodies are not commercially available, antibodies were raised in rabbits, following a double-step chromatographic purification of Vtg from plasma of E2-treated male sea bass [4]. Statistical analysis was performed using Dunnett's test or Tukey–Kramer method as appropriate (Prism software). The level of significance was set at $P = 0.05$.

RESULTS

Western blot analysis revealed the presence of signal in NP (50 mg $kg^{-1}$) and E2 plasma samples, where homologous polyclonal antibodies recognized almost five bands, with a major band at about 180 kDa. On the contrary absence of Vtg cross-reaction was observed in plasma of negative controls and NP-treated at the lower dose. The indirect ELISA showed that E2 increased the Vtg levels approximately 550-fold compared to negative controls. NP at 50 mg $kg^{-1}$ induced a 2-fold increase of Vtg levels while the lower dose was ineffective.

DISCUSSION AND CONCLUSIONS

Both in Western blot and ELISA assays our homologous polyclonal anti-sea bass Vtg antibody revealed a good level of cross-reactivity against sea bass-Vtg, while commercial available heterologous antibodies (salmon, carp, seabream, turbot) show none/weak cross-reactivity (unpublished data). NP showed a weak oestrogenic effect in immature sea bass compared with the strong response reported in other fish species [5].

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## G-21 (0232)

## Poisonings in animals: the 2000–2002 report of the Poison Control Centre of Milan

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INTRODUCTION

The Poison Control Centre of Milan (CAV) receives each year the 64% of the total national calls, for human exposures, of toxicological consulting in Italy. A previous work [1], a 1991–2000 report of the CAV, recorded about 2350 animal intoxications. Unfortunately not specific information could be collected at that time. Actually an 'active' collaboration with our University permitted, with a continuous follow up, a more extensive documentation of reasons of exposure, duration of toxic effects, therapies used and clinical effects. From 2000 to 2002 the CAV collected 430 animal exposures, and more than 78% of the total