WILD BIOLOGICAL REAGENTS AND DIOXIN RISK ASSESSMENT

S. Garagna, M. Zuccotti, M. Vecchi, P.G. Rubini, R. Fanelli*, C.A. Redi

Laboratorio di Biologia dello Sviluppo, Dipartimento di Biologia Animale, Universita' degli Studi di Pavia, Piazza Botta, 9 - 27100 Pavia, Italy
* Dipartimento di Scienze Ambientali e Sanitarie, Istituto di Ricerche Farmacologiche "Mario Negri", Via Eritrea, 62 - 20157 Milano, Italy

Introduction

Seveso, 16 km north of Milan, Italy, acquired worlwide notoriety on 10 July 1976 because a vapour cloud containing at least 2 kg of 2,3,7,8-tetrachlorodibenzopara-dioxin (TCDD) issued from a reactor producing trichlorophenol. The most polluted area (about 43 ha) was artificially reconstructed and transformed into a wood composed mainly of oaks with some scattered green fields and some bushy areas, the Bosco delle Querce urban park. The park has been colonized by annelids, insects, amphibians, reptiles, birds and mammals. Substantial populations of insects (both of the ground-dwelling, ambulatory community and flying community) and mammals (notably rabbits and house mice) are useful biological reagents for risk assessment because of their long-term exposure to TCDD. The 1998 TCDD soil concentrations do not exceed 16 pg/g dry soil, with no differences between the topsoil and 15 to 30 cm deep; the concentrations in moss and earthworms were 5 to 25 pg/g and the air concentrations of 15 TCDD isomers investigated were 0.3 to 21 pg per cubic meter (1).

Materials and Methods

Wild houseflies, housemice and rabbits caught at the Seveso Park were employed to perform mutagenicity tests and study gross morphology features. These are endpoints of the action of xenoestrogen-like molecules such as TCDD, even at doses below those that exert maternal effects (2). These data were compared with those obtained on TCDD treated houseflies and housemice. A total of 43 male and 32 female houseflies were collected in the inner part of the Seveso park, where horses and pigs are kept. Males and females were kept in the housefly insectary, where the standard WHO (World Health Organisation) and several mutant strains are reared, and a new strain named "Seveso 96" was established by matching the 32 females with 10 males, both of Seveso origin. Some original Seveso males were coupled with females of the standard WHO strain to get small mass-bred progeny and then matched in a "single pair" way with marked females to search for sexual determinants.

The comet assay was employed for the detection of DNA breaks in individual sperm following the methodology described by Fairbairn et al. (3). Briefly, sperm isolated from vas deferens were suspended in low melting point agarose, smeared onto a slide and run under an electrophoretic field and stain with ethidium bromide. When compared with the appropriate control samples (untreated and X-ray treated sperm), the lenght of the fluorescent DNA comet represents an indication of sperm DNA damage.

Results and Discussion

In wild caught animals, fetuses (16 rabbits, 38 house mice) and newborns (9 rabbits and 17 house mice) do not show signs of TCDD action; the number of corpora lutea in pregnant females did not differ significantly from the number of living implants plus reabsorptions (which were very low and similar to those of the control animals caught in other urban and suburban parks). Male reproductive organs and the germ cells cytodifferentiative process were regular in 9 rabbits and 21 house mice: there were no lacuna between Sertoli and gonia cells and no vacuolization of Sertoli cytoplasm; the cell number and composition of the stages of the seminiferous epithelium cycle resulted in a regular histological architecture; a sperm morphology (mutagenic) test was not significant; the sperm aneuploidy rate was not significant and the sperm comet assay (showed DNA comets (in a low percentage, 3 to 4%, as in control animals) with a lenght similar to that of the control sperm than that of the sperm radiated at a final dose of 8 rad. The number of bone marrow micronucleated polychromatic erythrocytes (MPCEs) per 1000 PCEs (a mutagenic test) was never higher than 0.74±0.27 in any experimental group of the wild caught animals. The data obtained from house mice exposed to 100 pg/g up to very high dosages of TCDD differed dramatically and all the test performed, including the gross morphology analysis, clearly showed as dioxin can impair germ cell, embryos and fetuses development. Comparing these last data with those of the wild biological reagent from the Seveso park, it can be suggested that the biological risk for TCDD at the Seveso park does not differ significantly from that of the other parks investigated. This conclusion is supported by the TCDD liver concentrations of the animals from the Seveso park: 4.3±0.4 versus 7.2±2.9 pg/g of fat and 29.5±13.8 versus 41.3±9.5 for Seveso versus controls in rabbits and house mice, respectively.

Particularly noteworthy, the genetics of the housefly gave quite interesting insights. All houseflies caught in the park were morphologically normal while two F1 females (1 from an outcross and 1 from an inbreeding) out of 400 flies scored showed abnormal tergites. It must be remembered that flies with abnormal tergites are very rare, sporadically occurring in mass breeding. The percentage of individuals with atrophic testes rises to 13% and 28% respectively in the progeny of the first and second outcross of the original Seveso males with standard marked WHO females. Among the F1 progeny of inbreeding crosses were recessive morphological mutants of the curly (cy) and divergent (dv) wing types and mutants with pigmented external tergites border, pigmented sternites (ps); the occurrence of these mutants is sporadic in laboratory strain mass-breding. We have not yet completed a similar analysis on wild flies from other urban and suburban park and thus any inference from these data must be extremely cautious. However, the possibility of establishing a very sensitive TCDD animal bioindicator is currently explored in the laboratory, by exposing flies to increasing concentrations of TCDD and looking to chromosome morphology. In fact, several chromosomal abnormalities were detected among wild and F1 Seveso 96 progeny: secondary constrictions, particularly frequent over chromosome 1 and 2 and over the long arm of chromosome 3; breakages and deletions are the chromosomal abnormalities we detected in both the homozygous and heterozygous state. It is particularly noteworthy that breakages and constrictions are still present in the third generation (from the original Seveso animals) and thus viable.

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