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Prenatal risks deriving from environmental chemicals

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Summary. - Hundreds of environmental chemicals affect prenatal development in experimental animals. However, only methylmercury and PCBs have been connected with such effects in humans during localized outbreaks of high exposure. In addition growth and development might also be affected by long-term intake of lead, fluorides or PCBs. Several factors may explain the discrepancy between human and animal data: the actual exposure of the population is below threshold levels, unspecific or delayed effects can be difficult to identify, etc. When experimental data are used to assess the hazards for the conceptus, due consideration should be given to actual ability of the study to detect effects. Thus, the limitations in statistical power, the relevance of the parameters considered and low-dose extrapolation should be taken into account. Finally, understanding toxicokinetics and biological mechanisms is needed to perform interspecies comparisons. Three examples of environmental chemicals showing different prenatal hazards are presented: thiabendazole, a benzimidazole compound with a moderate teratogenic potential, but which could represent a good model for biological extrapolation; nitrofen, a diphenyl ether herbicide which may pose a significant hazard, because of its high potential, toxicokinetics, and specific, hormone-like, teratogenic mechanisms; PCBs, well-known, global, cumulative pollutants which are not teratogenic in the laboratory animals, but may affect the human conceptus at high intake levels.

Key words: prenatal toxicology, environmental contaminants, thiabendazole, nitrofen, polychlorinated biphenyls.

Riassunto *(Rischio prenatale indotto da inquinanti ambientali)*. - Centinaia di sostanze chimiche presenti nell'ambiente hanno una dimostrata tossicità prenatale negli animali da laboratorio. Tuttavia nell'essere umano questo effetto è stato dimostrato solo per il metilmercurio e i PCB, in situazioni localizzate di alta esposizione. Inoltre, anche assunzioni croniche di piombo, fluoruri o PCB potrebbero avere un impatto sulla crescita e sullo sviluppo. Svariati fattori possono spiegare la differenza fra i dati sperimentali e quelli umani: i livelli ambientali di esposizione sono inferiori ai livelli soglia per l'insorgenza di un danno prenatale; effetti aspecifici o ad insorgenza ritardata possono essere di difficile identificazione, ecc. Quando si utilizzano dei dati sperimentali per caratterizzare il rischio prenatale, occorre considerare la effettiva capacità dello studio di identificare gli effetti. Sono perciò importanti le limitazioni statistiche, il tipo di parametri valutati e la estrapolazione alle basse dosi. Infine, la comprensione della tossicocinetica e dei meccanismi di azione sono necessari per comparare i risultati fra specie diverse. Vengono presentati tre esempi di composti chimici ambientali dotati di differenti potenziali di tossicità prenatale: tiabendazolo, un composto benzimidazolico con un moderato potenziale teratogeno, ma che potrebbe rappresentare un modello interessante di estrapolazione biologica; nitrofen, un difenil etere impiegato come erbicida che può porre un rischio significativo, a causa del suo forte potenziale, tossicocinetica e meccanismo teratogeno specifico, simil-ormonale; PCB, contaminanti ambientali ad azione cumulativa e a vasta diffusione che non sono sperimentalmente teratogeni, ma possono danneggiare il prodotto del concepimento umano ad alti livelli di esposizione.

Parole chiave: tossicologia prenatale, contaminanti ambientali, tiabendazolo, nitrofen, bifenili policlorurati.

Introduction

It is generally recognized that within a 2-3% "baseline" prevalence of birth defects in humans, only 5-10% are surely attributable to extrinsic causes and half of them to drugs and chemicals [1, 2]. However, about two thirds of birth defects are suspected to arise from an interplay of factors. In particular, such comparatively frequent malformations as neural tube defects, cleft palate/cleft lip, heart abnormalities or talipes equinovarus are attributed to multifactorial inheritance, i.e., to a polygenic predisposition interacting with environmental triggers [3].

Very few environmental agents are known to actually affect the human conceptus [2]. In isolated situations of very high exposure, methylmercury induced severe congenital brain damage [4] and polychlorinated biphenyls (PCBs) a specific pattern of fetal intoxication [5]. There is also evidence that chronic exposure to such chemicals as lead [6], PCBs [7] and fluorides [8] may

interfere with human development. Sadly, the teratogenic risks secondary to the use of chemical weapons would also deserve consideration: this aspect is briefly reviewed by Schardein [2] and it will not be dealt with in the present paper. On the other hand, developmental toxicity in laboratory animals is usually detected by conventional "teratogenesis" (segment II) studies and, to a lesser extent, multigeneration tests [9, 10]. By means of experimental studies over 800 chemicals (including many pesticides, industrial by-products, etc.) have been recognized as "teratogenic" [1, 2]. Such discrepancy in sensitivity between humans and laboratory animals might be due to a greater resistance of our species. However, it is more likely to arise from the difficulties of detecting effects in the usual "field" situations (i.e. exposure of an heterogenous population to ubiquitous traces of multiple xenobiotics).

Apparently, no large epidemics of birth defects have occurred in our species in the last decades, in spite of growing exposure to environmental chemicals and to growing concern for the possible health effects [2]. It should be kept in mind that for most true malformations an exposure sufficiently high must occur during the narrow window of sensitivity represented by the organogenesis period: in the woman such rised between gestational days (GD) 15-20 and 50-60 [11]. However birth defects include as well those insults to growth and/or development which may be induced in the fetal and even the perinatal periods and which are surely more difficult to study.

The following paragraphs will present three examples of chemicals causing concerns because of their prenatal toxicity potentials, i.e., thiabendazole, nitrofen, and polychlorinated biphenyls. The available data will be summarized in order to identify and characterize hazards.

Thiabendazole (TBZ)

TBZ is a benzimidazole compound with high, broad-spectrum fungicide activity: like others benzimidazoles, TBZ reversibly inhibits the polimerization of tubulin [12]. It degrades slowly in the soil and residues in the range of 10 ppm have been detected on citrus fruits: human oral exposure is thus probable [13]. The first studies performed on reproductive/prenatal toxicity have been concisely reported in [12]. No effects were seen in a 5-generation test in the mouse and in a 2-generation test in the rat; also, no clear-cut effects were observed in four oral teratogenicity (exposure on GD 8-16) studies in the rabbit, but for a small, dose-related increase of unspecified "abnormalities" at 400 mg/kg b.w. At the highest dose level of 800 mg/kg b.w. only 40 fetuses were examined, a quite unsufficient sample.

When pregnant rats were administered *per os* to 125, 250 or 500 mg/kg b.w. of TBZ suspended in distilled water on GD 6-15, no signs of maternal toxicity were elicited. Modest, but significant and apparently dose-related effects were observed in the litters, namely: reduced fetal weight at 125, increase of resorptions at 250, and increased prevalence of fetuses with abnormalities at 500 mg/kg b.w.; the prevalence of litters having ≥ 1 fetus with abnormalities was increased at all dose levels. Actually, the reported "abnormalities" were indicators of developmental delay (i.e., runting and/or poor ossification of sternum), not malformations. Nevertheless, a NOEL could not be determined in this segment II study on the rat [13].

A teratogenic potential was consistently identified in the mouse during a series of studies performed by Japanese toxicologists. TBZ dissolved in olive oil reached higher and faster peak levels in the plasma, embryos, fetuses and placenta, as compared to TBZ dissolved in 0.5% arabic gum. Moreover, when 1000 mg/kg b.w. TBZ were given *per os* in olive oil on GD 9, effective concentrations were present in the embryonic compartment in the first 6 h following treatment; only trace levels were present after 72 h [14]. Three experiments aimed to identify the critical periods for the effects and the dose/response curve were then performed on the Jc1:ICR mice using olive oil as vehicle. The findings can be summarized as follows [15]:

- treatment on GD 6-15 with 700, 1300 or 2400 mg/kg b.w. caused high maternal toxicity and a dose-related increase of specific abnormalities. At the lower dose a significant reduction in dam's weight gain was observed, without increased maternal or intrauterine mortality: cleft palate and vertebral fusions were observed in 8% and 1% of fetuses, respectively;

- a single 2400 mg/kg b.w. dose during one day within GD 6-15 caused maternal mortality and a malformation pattern dependent on the day of treatment (from exencephaly on GD 6 to cleft palate on GD 13), suggesting that TBZ is a general, unspecific teratogen. The highest overall increase in malformations was obtained on GD 9: this was also the main critical day for limb reduction deformities, which had never observed before in the historical controls of the strain employed;

- the dose-response curve was studied in mice treated with 30-2400 mg/kg b.w. (17 dose levels) on GD 9. Maternal mortality and reduced b.w.gain were elicited by dose levels ≥ 1667 and ≥ 1157 mg/kg, respectively. The prevalence of resorptions increased only at ≥ 1667 mg/kg, but fetal weight was significantly reduced even at ≥ 60 mg/kg b.w. The treatment did not increase the prevalence of malformations other than reduction deformities of limbs at ≥ 485 mg/kg b.w. and vertebral fusions at ≥ 60 mg/kg b.w. The abnormalities of tail and/or anus increased, too, but the data were not reported. The ED_1s for limb and vertebral malformations were obtained according to regression analysis/probit response and were 362 and 26.4 mg/kg b.w., respectively. The authors observed that the ED_1 for vertebral abnormalities

- considered as the highest nonteratogenic dose - is 500 times the A.D.I. of TBZ used as food preservative (0.05 mg/kg b.w.). However, the ED_1 could be exceeded when TBZ is used as anthelminthic in human medicine;

- finally, the findings from some experiments performed on other species were also summarized. The oral treatment of rats on GD 8-15 with 296-1000 mg/kg b.w. of TBZ suspended in olive oil induced a dose-related increase of litters with ≥ 1 fetus having generalized oedema. When a single 1000 mg/kg dose was administered to rats during one day within GD 8-15, only isolated cases of abnormalities were observed: they were nevertheless consistent with those previously obtained in the mouse.

A comparable study in the rabbit did not induce any apparent, treatment-related abnormality.

The toxicokinetics of TBZ in the maternal and embryonic compartments was investigated in greater detail [16]. Upon a single 1000 mg/kg dose on GD 13 the total radioactivity peaked after 1 h then declined. However, bound residues reached peak levels in 3-24 h, either in maternal tissues and in the embryo, and declined slowly, as small amounts were detectable even after 1 week. When TBZ was administered on GD 13 at 200-1600 mg/kg b.w., the bound residues showed a dysproportional, markedly nonlinear increase at the higher dose, suggestive of an altered metabolism. The authors found also a significantly positive relationship between the dose/response curve of binding to fetal macromolecules and the number of skeletal malformations per fetus (though not with the prevalence of affected fetuses). They assumed that cell death from irreversible covalent binding could reach a critical level leading to the malformation pathway with a dose-related increasing frequency [17].

According to studies performed *in vivo* and *in vitro*, TBZ-induced limb reduction deformities can derive from the inhibition of ATP synthesis in limb buds. Mouse embryos exposed *in utero* on GD 9 to 1000 mg/kg had ATP levels in limb buds which were 33-40% of control values after 24 h. Rats similarly treated on GD 11 had levels 80% of control values: such difference was attributed to the higher (about 2-fold) physiologic levels of ATP in this species. At the ED_1 level for limb abnormalities in mouse (362 mg/kg) [15] the ATP in limb buds was 68-75% of controls and the authors thus assumed that limb reductions may be elicited when levels show a >30% decrease. Noticeably, when mouse embryos were exposed to 1300 mg/kg in 0.5% arabic gum the ATP values were 91-94% of control values [18]. In mouse limb buds coltured *in vitro* the inhibition of proteoglycan synthesis occurs at lower concentrations, and to a greater extent, than inhibition of DNA synthesis and cytotoxicity [19].

In summary, TBZ is an interesting item for research on teratogenetic mechanisms but is unlikely to pose any significant risk for human development at environmental exposure levels. However, such compound may deserve further attention as a model of hazard assessment based on biological mechanisms. A reasonable curve is in fact available for extrapolation to low dose levels with regards to major effects [15, 18]: informations on the levels of ATP in human embryo limb buds and on the toxicokinetics of TBZ in humans, expecially about the presence and persistence of bound residues, could be compounded to fully characterize the level of hazards in the human conceptus.

Nitrofen (NF)

NF (2,4-dichloro-4'-nitrodiphenyl ether) has been developed as preemergent and postemergent herbicide: its oral LD50 in the rat is 2.4-3.6 mg/kg, and it increases liver weight and induces citochrome P-450 upon subchronic administration [20]. NF is recognized as a potent animal teratogen acting through specific mechanisms. In fact, it can induce a characteristic pattern of soft-tissue malformations both through oral and dermal exposure and a NOEL has not been demonstrated in the rat: levels as low as 0.3 mg/kg b.w. during the organogenesis period still increase the prevalence of hydronephrosis [20, 21].

Following absorption, NF distributes to a variety of tissues, reaching the highest concentration in the gonads and fat. It is not readily detoxified through biotrasformation and the half-life for excretion may be as long as 6 days. NF can be released from depot tissues again in the bloodstream and redistribute: such recirculation certainly contributes to the teratogenic potential observed at low dosages. It accumulates progressively also in the embryonic compartment where, however, the actual amount is low and comprises only a small fraction of the total AUC [20, 21].

The teratogenetic mechanisms of NF have been extensively reviewed in [20]. The spectrum of NF-induced effects includes abnormalities of heart (trasposition of great vessels, ventricular septal defects), kidney (hydronephrosis), lung (hypoplasia), diaphragmatic hernia and hypoplastic/absent harderian glands. Such pattern can be induced by a single 75 mg/kg b.w. dose and GD 10 appears as the most critical period in the rat. Overt maternal toxicity, or significant increases in prenatal mortality or external malformations are not observed: most affected pups, instead, develop respiratory distress and die shortly after birth. It is also noteworthy that NF showed the same teratogenetic potential in in SD and Long Evans rats, but the patterns of abnormalities were somewhat divergent, suggesting a different embryonic susceptibility in the two strains. The

NF-induced syndrome may arise from an alteration in the thyroid hormone status leading to an increased embryonal availability of free hormone. Alternatively, NF itself can directly cause a premature thyromimetic challenge leading to an altered differentiation.

Diphenyl ethers are a major class of herbicides and have also a wide range of industrial usages. The role of the number or position of C1 substituents on the maternal and developmental toxicity of several diphenyl ether analogs of NF was assessed in CD-1 mice to investigate structure-activity relationships. None of the analogs was as active as NF with respect to the parameters assessed (liver weight in dams, total litter loss, postnatal survival, small/absent harderian glands). The number and position of C1 substituents actually bore some relationship with the toxic effects; it was impossible to detect, however, any simple correlation [22].

Several papers dealt with the biochemical and/or functional postnatal consequences of prenatal NF exposure. CD rats were treated *per os* with 12.5 or 25 mg/kg b.w. on GD 8-16: almost all selected biochemical indicators of brain, kidney, liver and lung development were affected in fetuses examined on GD 18-21. Dose-related reduction of fetal weight and increased prevalence of diaphragmatic hernia were also induced by the treatment. However, such effects as decreased lung weight and phospholipid content and - at the higher dose level - decreased liver weight were considered specific (i.e., not due to fetal growth retardation), as they persisted after covariate analysis [23]. Renal effects were studied in the offspring of Long Evans treated with NF on GD 6-15. The higher dose level, 25 mg/kg b.w., caused a 100% mortality in the first two days of postnatal life. At 6.25 mg/kg, body weight was unaffected on GD 20 and was reduced by about 5% compared to controls at weaning: harderian gland development - considered as a pathognomic sign - was affected in 11% of pups. Reduced papillary length and increased dilatation of renal pelvis and tortuosity of ureters were observed, the prevalence of absent pelvis being more than double in weaned pups than in term fetuses. The ability to concentrate urine was reduced when assessed on postanatal day (PD) 9-10: such effect was considered as related to the persistent retardation of papillary and ureteric development [24]. Pregnant SD rats were treated with 50 mg/kg b.w. on GD 11: the findings confirmed that the prevalence of hydronephrosis in pups increases with age (from 4% on PD 7 to 20% on PD 29). The growth of hydronephrotic animals was almost unaffected: however, their poor urine concentrating ability was confirmed, and it worsened after weaning (i.e. after changing to a more osmotic diet). Urine concentrating ability was reversibly reduced even in pups with apparently normal kidneys [25].

The apparently specific effects of NF on lung growth were investigated in newborns from rats treated *per os* with 20 or 40 mg/kg b.w. on GD 10-13. Both dose levels affected lung weight and volume more than general growth: in particular, at 40 mg/kg there were a 50% reduction in the number of airspaces and marked deficits in functional tests. Pneumocytes appeared more simple and immature, and showed reduced contents of DNA, RNA, proteins and regulatory polyammines. Interestingly, at both dose levels there was a significant reduction in adrenal epinephrine, critically involved in the transition to air-breathing of the neonatal lung. The authors concluded that respiratory distress is a major factor in the neonatal mortality of rats prenatally exposed to NF [26].

The spectrum of postnatal sequelae was investigated in the offspring of CD-1 mice treated on GD 7-17 with dose levels ranging from 6.25 and 200 mg/kg b.w. No apparent maternal toxicity was observed. The harderian gland appeared as the primary target organ: a 4% prevalence of absent gland was observed at a dose as low as 25 mg/kg and the average weight of the glands was reduced even at the lowest dose level. At 6.25 mg/kg lung and liver weight were also significantly lower than in controls. Such effects were more sensitive indicators than reduced postnatal growth, which was bserved at $\geq$ 12.5 mg/kg b.w. and were considered difficult to detect in the conventional teratogenicity tests. In particular, the absence or hypotrophy of harderian glands manifests itself during eye opening, and especially after PD 12-13 when the glands start functioning. Impaired reproductive development was also present, as delayed puberty and reduced fertility were detected in females at $\geq$ 50 mg/kg b.w. At this dose level there was also increased postnatal mortality, which showed a very steep dose-response curve and reached 100% at 150 mg/kg. Interestingly, no effects on kidney development were present and the prevalence of diaphragmatic hernia was only 6% at the highest dose level. A cross-fostering experiment using 100 mg/kg b.w. showed that the effects were due solely to prenatal exposure [27].

NF was examined with other pesticides in a scoring system aimed to rank chemicals with regard to risks of developmental toxicity [21]. NF is rapidly absorbed through the skin, showing a high percutaneous bioavailability: in fact, blood levels equivalent to those found after i.v. injection can be reached through percutaneous transport. Considering the lack of a developmental NOEL and that the hazard for workers would not be adequately mitigated by conventional protective measures, it is not surprising that NF scored higher than such compounds as ethylenethiourea or dinocap. In fact the chemical is no longer registered in the USA.

To summarize, NF is a teratogenic agent acting through specific mechanisms and inducing a range of abnormalities of differentiation which may not be fully detected by the conventional segment II test.

Considering its developmental toxicity potency and toxicokinetics and bioavailability characteristics, NF might pose a significant risk at relatively low levels of environmental exposure.

Polychlorinated biphenyls (PCBs)

PCBs were marketed from the 1930s as industrial chemicals to be employed where highly stable fluids were required. In 1976 their production was banned in the USA: it is estimated that more than 400,000 t had been produced in that country and that 80% were released in the environment.

PCBs are outstanding global pollutants, due to their poor degradation and potential for biomagnification. They are absorbed through ingestion, skin contact and inhalation, accumulate in the adipose tissue over lifetime and show a significant excretion only during lactation: detectable levels are usually found in humans, mammals, birds and fishes [5, 7]. It should be kept in mind that the widely used commercial preparations were manufactured as mixtures containing up to 50 of the possible > 200 isomers as well as traces of toxic impurities (PCtri- and -quadriphenyls, and PCdibenzofurans -PCDFs) [7].

According to studies performed on rodents, PCBs may induce general toxic effects in the offspring; however, they do not elicit an increase in birth defects. The distribution of C^{14}PCB was studied in mice injected im on GD 14 or 18. The uptake by the fetus was very low, but increased one day before parturition. The radioactivity was predominantly in the intestine and urinary bladder, although lower amounts were detected in the liver and kidneys, and also in the yolk sac and placenta: these were deemed as possible sources of fetal uptake [28]. No apparent effects were elicited in a 9-week, 2-generation study using 70 ppm (6.4 mg/kg b.w.) Aroclor 1254 in the drinking water to Wistar rats. The placental barrier acted quite effectively, as the PCB content in GD18 fetuses was low; due to trasfer through the milk, the levels in sucklings were higher [29]. The placental trasfer is limited by the high uptake by the maternal adipose tissue and by the rapid excretion in the feces. Differences may exist between PCBs, as those with higher C1 content can have a faster placental trasfer. Maternal milk offers a substantially greater exposure: in rats < 0.2% of the total dose passes to fetuses, but ≤ 2% to pups up to weaning [7]. Adverse effects were detected in a more recent study, where female rats were fed 2.5, 26 or 269 ppm Aroclor 1254 from mating through the weaning of the pups. Pup mortality was markedly increased at the highest dose levels. Dose-related reduction of pup weight gain and delayed reflex ontogeny were observed at all dose levels, as well as increased liver weight and decreased spleen and thymus weight. As shown by PCB levels determined in the brain, the mother to conceptus trasfer was greater during lactation than during gestation: significant residues were also detected in the brains of perinatally exposed adult rats [30]. In a multigeneration study in the rat, dietary exposure to > 5 ppm Aroclor 1254 reduced both litter size and pup growth, and even a lower exposure increased the pup relative liver weight. On the other hand, increased liver weight and enzyme induction were observed, both in dams and pups, only when levels >100 ppm of Aroclor 60 were fed [7]. When pregnant guinea pigs were administered a total oral dose of 100 mg (2.2 mg/day) of Clophen A on GD 16-60 a severe prevalence of fetal death was observed [31]. Pregnant rabbits were fed 10 mg/kg b.w. Aroclor 121 or 1254 on GD 1-28. Placental trasfer occurred for both mixtures; however no effects were detected in fetuses, but for a decreased liver vitamin A content in the Aroclor 1254 group. In fact, increased liver weight and enzyme induction were observed in the dams, not in the offspring [32].

PCBs were also observed to induce a range of effects in the mouse, which deserve mentioning, although they appear species-specific and/or dependent from the high doses or the administration routes employed. An irreversible neurological syndrome was induced after exposure to 3,4-3'4'-4CB on GD 10-16; the likely mechanism was an impaired development of the dopaminergic system [33]. A specific teratogenic (mostly cleft palate) effect was observed in ddY mice injected sc with PCB in 0.05 ethanol during the organogenesis [34]. Oral treatment with 375-500 mg/kg b.w. of 2,2'-2CB on GD 1-3 delays implantation [35].

Some other findings may be more relevant to hazard characterization, as they provide some evidence of functional impairment in the offspring. Lactating mice were treated with 4 weekly injections of 50 mg/kg b.w., from the day of delivery: the treatment slightly but significantly reduced the reproductive capacity of the offspring of the treated animals [36]. Pups from NZW rabbits fed 250 ppm PCB 4 weeks before mating through pregnancy and lactation showed a significantly reduced contact sensitivity response when tested on PD 49. However this effect could be unspecific as lower b.w. weight and increased liver weight were also present at 250 ppm; moreover, at 100 ppm, hepatic microscopic alterations, but no immunological impairment, were detected [37].

The fetotoxicity of PCB has been studied also in the rhesus monkey. As in rodents, PCB accumulation increases during the late fetal period, particularly in the dermal tissue where fat depots start to form [7]. When 630 or 3150 μg/kg b.w. of 3,4,-CB were given in 9 divided doses between GD 20 and 40 (equivalent to 2 and 10 ppm in a continuous feeding exposure), abortions and fetal deaths were induced. Maternal death did not appear or appeared only after abortion: as the fetal rhesus becomes independent from maternal progesterone on GD 20, the effects was likely to be direct [38]. When

rhesus females were fed 2.5 ppm PCB for 6 months, their offspring had low birth weight and focal skin hyperpigmentation; abortions and fetal deaths were also frequently observed [7].

Two well-known mass intoxications were caused by PCB-contaminated rice oil brands in Japan (1968, "Yusho") and Taiwan (1979, "Yu-cheng"). Between 3000 and 5000 people were involved overall; 40-60 babies were born showing a characteristic intrauterine intoxication with an attack rate of 3.9% on exposed pregnancies in Yusho [5, 7, 39]. The fetotoxic effects paralleled maternal exposure to PCBs: mothers with no symptoms gave birth to mildly intoxicated infants, while severely symptomatic mothers had stillbirths [39, 40]; overt anomalies were present with a maternal intake of ≥ 70 μg PCBs and 0.4 μg PCDFs per kg b.w./day [7]. However the actual exposure was impossible to measure and blood levels were unreliable indicators due to PCB toxicokinetics; thus, a dose/effect relationship could not be established [5]. In the Yusho outbreak at least 13 cases were recognized arising from the residual maternal body burden: possible mild cases were reported up to 1973 [40]. The main features of Yusho were deep brown ("cola-coloured") skin and mucosae which reverted to almost normal in 2-5 months. Other features were not present in all cases and included natal teeth and gingival hyperplasia; hypoplastic pigmented nails; poor cranial ossification; cystic alterations of eye glands; marked facial edema; liver toxicity as shown by elevated enzyme levels, histopathology and/or hepatomegaly [5, 39, 40]. Two out of the 8 described cases of fetal Yu-cheng showed acneform eruptions, but no teeth or gingival abnormalities were recorded [5, 40]. In both outbreaks a high incidence of fetal loss [7] and a significant intrauterine growth retardation were also reported; the latter did not fully reverse postnatally [39, 40], possibly due to postnatal exposure [7]. Alltogether, the most characteristic features suggested an enzymeendocrine disturbance, including also irregular calcification [39, 40]. The total amount of contaminant ingested was about the same in Yusho and Yu-cheng; thus a different composition of the PCB mixtures involved might have caused the observed clinical discrepancies between the two outbreaks [7]. Actually, at least part of the symptoms could have derived from such PCB impurities as PCQs or PCDFs: these compounds have markedly higher toxic potentials than PCBs [5] and their tissue levels were much higher in adult Yusho patients than in workers occupationally exposed to PCBs [7]. Finally, developmental delay and neurobehavioural problems were reported in Yusho children reexamined when 7-9 years old [5] and in a cohort of 117 exposed children in Taiwan [7]. In Japan, according to a nationwide monitoring, female newborns or infants had higher prevalences of reduced intrauterine and postnatal growth when the mother's milk fat had ≥ 1 ppm of PCB: this finding was confirmed after matching for confounding factors and was not detected in males [39]. Although these data could have some biases, they suggest that unspecific developmental alterations might derive from exposures to low levels of PCBs intake. In the USA, mothers recalling a strong consumption of fish from PCB-contaminated rivers and lakes were reported to have infants lighter and with smaller head circunference than controls [7].

Human environmental exposure to PCBs is likely to continue for long time: high accidental exposures from poorly controlled disposal of contaminated equipment are also likely to occur, particularly in developing countries. A pregnant woman may have blood PCB levels in the measurable ppb range, regardless or any special intake, and "threshold" or "normal"values are not available; however, no reports of fetopathy exist outside the two outbreaks in Eastern Asia [5]. An estimation of birth defect hazards due to pyrolysis by-products of PCBs (i.e., mainly PCDFs) has been performed by means of Monte Carlo analysis: the risk is reported as negligible, when the exposure dynamics are taken into account [41]. In the USA the highest exposure comes from breast-feeding, and after weaning, from sport fish from contaminated waters: the American Academy of Pediatrics recommended assaying breast milk, and discouraged eating sport fish during pregnancy, as well as dieting as chemicals might be mobilized from fat depots [5].

To summarize, the environmental levels of PCB contamination are unlikely to increase significantly the risk of birth defects. However, at the upper range there could be an unspecific impact on reproduction and on the development of the offspring.

Discussion

With regards to the assessment of prenatal risks from environmental contaminants, several problems have to be considered. The following paragraphs will deal with a few of them.

Segment II studies

At present, conventional segment II studies are the best tool to study effects on the whole organogenesis [42]. Multigeneration studies are a highly valuable method in oder to detect a general impact on the general parameters of viability, growth and development of the offspring upon chronic exposure; however, they do not provide a characterization of the effects [10].

With regard to the use in risk assessment of segment II studies (or of toxicological tests whatsoever) their limitations in statistical power must be taken into account. Major malformations can be insensitive indicators as large sample sizes may be required to detect an effect unless the compound is a potent teratogen [9, 43]. The same applies, although to a somewhat lesser extent, to prenatal mortality. Fetal weight - a variable continuously

distributed within a relatively narrow range - is the most sensitive endpoint: a ≥ 10% change can be detected with a sample of 20 litters/dose level [9, 43]. Selecting appropriate dose levels is also relevant; an appropriate LOEL should approach the "threshold", i.e., slightly increase the background prevalence of effects and/or induce potentially reversible effects only [9].

Finally, the actual biological importance of the findings may be difficult to evaluate, as in the case of "minor anomalies" [44]; moreover, the parameters evaluated in the conventional protocols may be unable to cover developmentally relevant endpoints [23-27].

Thresholds

It is widely recognized that teratogenic effects have biological threshold. In fact, a single dose exposure in the appropriate critical period is often more effective than a chronic low level intake, as it is more likely to overcome both the detoxifying capability of the mother and the repair potential of embryonic tissues [42, 45]; it is also likely that genotoxic -"no-threshold"- changes in somatic embryonic cells play a minor role as teratogenetic mechanisms [45]. At population level a pragmatic threshold is what is needed for regulatory purposes. Nevertheless, a real threshold may be difficult to define, expecially with teratogens that interact with already existing mechanisms - or factors - that produce malformations (e.g., alcohol-induced fetal effects, compounds with hormone-like or hormone-altering activities). It may also be possible that, in a given population, individual threshold doses change along the time depending on whether other "environmental" exposures occur [46].

Finally it is possible that, within a population exposed to an agent, a subgroup is present which is particularly susceptible and/or is exposed in the most critical period of development. The actual effect on this subgroup showing a lower threshold might be absorbed by the background of the general population [47].

Low-dose extrapolation

The problem obviously arises as in most cases the experimentally effective dosages are significantly above the expected environmental exposure.

Several examples of mathematical dose-response models have been developed with regards to developmental toxicity, although they do not seem to be widely applied as yet. The "Hockey Stick" regression method, assuming a background frequency of effects, was utilized to assess the relationship between body burden and CNS abnormalities in infants prenatally exposed to methylmercury. A practical body burden threshold was demonstrated, above which the dose-response was approximately linear [48]. The modified Rai and Van Ryzin model takes into account the risks for malformations and prenatal death on a individual fetus, intralitter prevalence, and prevalence of affected (with ≥ 1 fetus affected) litters basis. According to such model, the risk to the litters shows small, progressive increments with the increasing dose, while the risk to an individual litter member increases significantly only at the high dose range [49]. The "benchmark dose" method attempts to improve two shortcomings of the conventional - NOEL plus safety factor - ADI approach: namely, the lack of criteria to define NOELs, especially with regard to "spontaneously" occurring lesions, and the little role given to the dose-response curve. The "benchmark dose" is defined as a statistically lower confidence limit to a dose produced some predetermined increase in the response rate (e.g., 1%). A well conducted experiment which does not define a NOEL could, thus, be acceptable for calculating the "benchmark dose" [50]. Low-dose extrapolation in developmental toxicity is also complicated by factors related to interspecies extrapolation. For instance, the actual fraction of the administered dose reaching the conceptus should be known [51].

Finally, one should mention some experimental systems that use critical embryonic periods to study the effects of low levels of radiations (and, possibly, of environmental agents in general) in the mouse. Endpoints are the mitotic delay of telencephalic cells, irreversible oocyte depletion in the neonatal period and the "homeotic shifts" of the thoraco-lumbar borders. Such tests were not designed to measure damage, but may be valuable indicators of an exposure to adverse factors [47].

Exposure of the human population

A careful exposure assessment is obviously of paramount importance to determine any risk from environmental chemicals. A detailed examination of this topics is outside the aim of this paper: to provide an example, with regard to pesticides the exposure of handlers, environmental inhalatory intake, exposure to residues in the field and in the various food items must all be taken into account [21]. Ingestion is the most frequently reported route of exposure in episodes of environmental contamination [52]. However, dermal or inhalatory exposure may be equally or more important, as assessed for organic volatile compounds in household water. If all exposure pathways are not considered, a significant underestimation of risks may result [53].

There are not many well-documented instances of environmental pollutants increasing the bith defect rate. No such increase was found after the notorious TCDD accident of Seveso, although a rise in the rate of spontaneous abortions was reported and there are still no information available on possible long-term effects [2, 54]. A primary item of exposure of the general population is represented by toxic waste disposal sites. According to data of the 1982, the USA industry generates 57-60 x 106 t/year of potentially wastes, 90% of which are disposed improperly. The case of Love Canal (USA, 1976-80) is

the most famous of such cases of environmental mismanagement. Several chemicals were involved, including TCDD. Although there were reports about increased rates of birth defects and miscarriages, no good studies were performed, especially with regard to delayed effects [2]. Specific, clear-cut prenatal damage was demonstrated in food contamination outbreaks involving methylmercury (Minamata bay, 1950s, and Niigata, 1960s; Iraq, 1971-72) [4] and PCBs [2, 5, 7, 39, 40]. Besides such outstanding, isolated cases of pollution accidents, only PCBs [7], lead and fluorides show an impact on development in humans at the high range of environmental exposure, albeit with a varying degree of evidence. Lead gradually accumulates in the fetus from GD 80-100 to term. Exposure to lead may interfere with pregnancy [2]; when Pb levels in maternal or cord blood are $\geq 100 \mu g/100$ ml, there is an increased risk of impaired CNS development [6]. Fluorine is taken up by the calcifying tissues in the fetus and may interfere with skeletal and teeth development in the early postnatal life [8].

The paucity of proven effects on the human conceptus must not remove the basis for concern; instead, it should support the need for investigation on less apparent or delayed developmental toxicity.

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