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Reproductive risks from contaminants in drinking water

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Summary. - Reproductive toxicity is a complex subject which, besides birth defects or sterility, includes adverse effects which may be less readily observed but more relevant to chronic low-level exposures (e.g., impaired functional development of target organs or systems, secretion of toxic chemicals in maternal milk). Sodium chlorite, when present at high concentration, may be related to impaired reproduction; among water chlorination by-products, further research is required on the developmental toxicity of chloroacetic acid and dichloro- and trichloro acetonitrile. Among the water pollutants which may pose significant developmental hazards, risk assessments have been performed for nitrates/nitrites, fluorides and lead. Molinate (a herbicide), dibromochloropropane (a nematocide) and the halogenated contaminants ethylene dibromide and epichlorohydrin show an almost selective male reproductive toxicity, although they are likely to pose a risk mainly at occupational exposure conditions. Ethylene thiourea, an environmental metabolite of some fungicides is markedly teratogenic in the rat: however, other toxic effects are induced at levels of exposure significantly lower than the teratogenic ones. Such poorly water-soluble compounds as TCDD or hexachlorobenzene also need to be considered, because of their remarkable potentials for reproductive toxicity. Finally, it should be noted that the data on reproductive toxicity for a number of chemicals do not allow a risk assessment at present: this holds true also for some substances which might pose concerns on the basis of their levels in drinking water (e.g. tetrachloroethylene, nickel). Another area which deserves more attention is the investigation of the possible interactions of several contaminants present together at low levels.

Key words: reproductive toxicity, herbicides, halogenated compounds, metals, nitrites/nitrates.

Riassunto (*Il rischio riproduttivo associato ai contaminanti delle acque potabili*). - La tossicità riproduttiva è un campo complesso che tra i possibili effetti, accanto alle malformazioni congenite o alla sterilità, ne comprende altri che possono essere sia meno visibili che più importanti per una esposizione cronica a bassi livelli di xenobiotici (es., alterazioni dello sviluppo funzionale di organi o sistemi bersaglio, secrezione di molecole tossiche nel latte materno). La presenza di alte concentrazioni di sodio clorito potrebbe essere associata ad effetti sulla riproduzione; fra i sottoprodotti della clorazione delle acque potabili, occorre effettuare ulteriori studi su acido cloroacetico e dicloro- e tricloro acetonitrile, in quanto potrebbero essere tossici per il prodotto del concepimento. Fra i contaminanti delle acque potabili dotati di potenziale tossicità dello sviluppo, sono state effettuate valutazioni del rischio per nitrati/nitriti, fluoruri e piombo. Il molinate (un erbicida), il dibromocloropropano (un nematocida) e i contaminanti alogenati etilene dibromuro e epicloroidrin mostrano una tossicità riproduttiva pressoché selettiva per l'apparato maschile, per quanto tali composti probabilmente pongano rischi soprattutto ai livelli di esposizione occupazionale. La etilenetiourea, un metabolita ambientale di composti fungicidi, mostra una chiaro effetto teratogeno nel ratto: tuttavia, altri effetti tossici si riscontrano ad esposizioni sperimentali nettamente inferiori a quelle teratogene. Pure certi composti scarsamente idrosolubili (es., TCDD o esaclorobenzene) devono venire considerati, a causa delle loro notevoli proprietà tossiche. Infine, occorre rilevare che per numerosi composti i dati di tossicità riproduttiva non permettono a tutt'oggi una valutazione del rischio: questo vale anche per alcune molecole che potrebbero destare preoccupazioni in relazione alla loro possibile presenza nelle acque potabili (es., tetracloroetilene, nickel). Un'altra area meritevole di maggiore attenzione è lo studio delle possibili interazioni di bassi livelli di contaminanti.

Parole chiave: tossicità riproduttiva, erbicidi, composti alogenati, metalli, nitriti/nitrati.

Introduction

Reproductive toxicity is a very complex process with multiple critical points. Besides birth defects and prenatal mortality, adverse effects on the conceptus can include alterations which are less readily appreciated, such as growth retardation or impaired functional development of organs or systems. Toxic effects on infants also deserve attention since some chemicals may be substantially excreted in mother's milk; babies might be more sensitive than adults and bottle fed infants may receive a higher exposure in relation to body weight. As for reproductive impairment, its impact may be reflected on the couple rather than on the individual (so theoretically affecting a portion of unexposed adults) [1, 2].

The risk of acute effects from drinking water pollutants is generally low, because concentrations of pollutants are usually very small; thus, the main concern regards the potential consequences of prolonged exposure, in particular when chemicals accumulate in the body [3].

The results of multigeneration studies (which provide an overall assessment of reproductive effects following long-term exposures) will therefore often appear more relevant to the potential risks from water pollutants than teratology studies which investigate the effects on the organogenesis following short-term exposure.

The US Environmental Protection Agency (USEPA) has considered the reproductive risks for a number of water contaminants of interest in preparing its Health Advisories (HA) [4]. These give estimates of the concentrations of chemicals anticipated to cause no adverse noncarcinogenic effect after a given exposure time. The role of reproductive toxicity data in determining some HA is considered here.

In addition, individual exposure may occur not only through ingestion of drinking water, but also through the skin and by inhalation [5]: so, data from inhalation or cutaneous exposure studies have been considered, when appropriate.

Reproductive risks from drinking water contaminants

Chemicals used in drinking water disinfection

Some reproductive effects have been demonstrated following exposure of laboratory rodents to *sodium chlorite* in drinking water. AJ mice showed lower female fertility and pup weight gain with 100 mg/l through gestation and lactation. In a one-generation study Long Evans rats showed decreased sperm motility and increase of abnormal sperms, while no effects were detected in the females and decreased postnatal serum levels of thyroid hormones were observed in the offspring (LOEL and NOEL: 100 and 10 mg/l, respectively). In another one-generation test in the SD rat, developmental delay was seen in fetuses: due to the small size of the experimental groups, the study could not detect sensitively whether low-frequency malformations were present or not (LOEL and NOEL: 10 and 0.1 mg/l, respectively). In humans a retrospective study compared two USA communities using chlorine dioxide or chlorination to disinfect drinking water: the latter had an excess of infants judged by the physician to be premature or to have a poor weight gain after birth. The rates of birth defects or neonatal mortality were unaltered; even though it has been experimentally shown that sodium chlorite induces hemolysis at exposures of ≥ 100 mg/l for ≥ 30 days, no increase of neonatal jaundice was found in this study. The average residual chlorine in drinking water was > 0.3 mg/l, decreasing with time to 0.1 mg/l. It is difficult to derive more than some suspicion from this study, as the parameters found to be altered were partly subjective, and a precise evaluation of them is generally difficult in a retrospective study [6].

Hypochlorite salts showed no effects in a 7-generation study performed in 1968 on BDII rats given 100 mg/l free Cl in drinking water: more recent data are lacking [6].

Chlorination by-products

CD-1 mice were given *bromoform* po for 18 weeks in a continuous breeding study starting 1 week before cohabitation: evident toxicity was seen in F0 and F1 (LOEL and NOEL: 100 and 50 mg/kg b.w., respectively) without signs of reproductive impairment or enhanced sensitivity of the offspring. In SD rats given po 50-200 mg/kg b.w. during organogenesis (i.e., on gestation days (GD) 6-15) a dose-related increase of minor skeletal changes was seen, without signs of maternal toxicity. Although the reviewers noted that in such study the examination of the soft tissues was insufficient, the available information does not indicate that bromoform has marked effects on reproduction [6]. The scant data available on *chlorodibromomethane* do not allow an evaluation, although they indicate that neonatal toxicity is present in mice exposed at ≥ 100 mg/l in drinking water [6].

A series of tests have been performed with *halogenated acetonitriles* (ACN) to screen prenatal toxicity potentials *in vivo* in the Long-Evans rats. Developmental effects were seen only at doses inducing maternal toxicity (50-55 mg/kg b.w. po) with bromo-, dibromo- and chloro-ACN: on the other hand, dichloro-ACN induced embryolethality, and trichloro-ACN malformations and embryolethality, at or below the NOEL for maternal toxicity, i.e., 25 and 15 mg/kg b.w. for dichloro- and trichloro-ACN, respectively. The corresponding prenatal toxicity NOEL were 15 and < 7.5 mg/kg b.w.: thus the risk for the conceptus arising from exposure to ACN may be somewhat related to the Cl presence in the molecule [6].

A slight increase in prenatal lethality, without apparent maternal toxicity was seen when 500 ppm *2-chlorophenol* in drinking water were administered to female rats from 10 weeks before breeding through gestation (NOEL: 50 ppm) [7]. Evident maternal toxicity and depression of birthweight were induced by ≥ 500 mg/kg b.w. *2,4,6-trichlorophenol* po to female rats (5 days/week) in a 2-generation study [8]. These data do not indicate that chlorophenols pose a significant hazard to reproduction in rats.

Chloramine did not elicit any evident reproductive impairment in a 2-generation study in the rat at doses up to 10 mg/kg b.w. po. Also, no adverse signs were detected in this study either in the adults and in the offspring [9].

Fu *et al.* [10] screened a series of seven halogenated water by-products *in vitro* by the hydra assay, whose endpoint is the Adult to Developmental toxicity ratio. Six of tested chemicals (dibromo-ACN; trichloro-ACN; 2-chlorophenol; 2,4,6-trichlorophenol; trichloroacetic acid; dichloroacetone) did not show an increased toxicity for the developing organism as compared to the adult.

Chloroacetic acid was > 8 times more toxic to the "developing" (i.e., regenerating) than to the adult hydra. The authors considered that, owing to such an unexpected potential, chloroacetic acid should have a high priority for *in vivo* investigations on developmental toxicity in mammals.

Other halogenated compounds

The following compounds cannot be evaluated because of lack of information: *chloroethane* [6], *cis and trans 1,2 dichloroethylene* [4] and *trichloroethylene* [4].

It is highly unlikely that significant risks for the conceptus will arise from *1,2 trichloroethane*, as maternal lethality was observed in absence of developmental toxicity in a screening study on mice [6]. Reproductive long-term studies have not been performed on this compound; however, *1,2 dichloroethane* did not induce any reproductive, developmental or general toxicity in a multigeneration study in ICR mice at exposures as high as 50 mg/kg b.w./day in drinking water [4].

Sufficient information is available on *1,1 dichloroethylene* [4]: this chemical did not show any specific effect on reproduction (at doses as high as 200 ppm - 26 mg/kg b.w. - in drinking water for 3 generations in the rat) and on organogenesis (at doses as high as 80 ppm by inhalation in the rabbit or 200 ppm in the diet in the rat). The studies performed on *tetrachloroethylene* (TCE) have shown some evidence of prenatal toxicity. When given by inhalation to rats and mice on GD 6-15 (300 ppm, 7 hour/day) TCE induced slight maternal toxicity and marked prenatal effects (two-fold increase in postimplantion loss, subcutaneous edema, minor skeletal anomalies); 900 ppm to rats on GD 7-13 or 14-20 altered brain neurotransmitter levels and behavioural performance in the offspring without causing histopathological lesions in the brain (NOEL: 100 ppm) [4]. Public health risks from *ethylene dibromide* (EDB) [11] derive mainly from its mutagenic/carcinogenic potential; however its effects on reproduction should not be overlooked. EDB was not teratogenic in standard segment-II tests, as the embryotoxic and maternal toxicity LOEL were the same (20 ppm by inhalation, to rats and mice). Kinetics studies in mice demonstrated a high concentration of the compound and its metabolites in the fetal respiratory and upper alimentary tracts: the sequelae of this finding in the postnatal life, if any, are not known. The exposure of female or male parents appears to produce different effects on the developing nervous system in the rat. Offspring performance in some behavioural tests was improved when females were exposed by inhalation to ≥ 6.67 ppm on GD 1-20 (NOEL 0.43 ppm): on the other hand, after po treatment of male rats with 1-1.25 mg/kg b.w. for 5 days impaired performance and changes in some neurotransmitter enzymes were observed in the offspring. Actually the main effect of EDB on reproduction is impairment of spermatogenesis. This effect has been widely investigated in ruminants (bulls and rams) as well as in rats. To summarize the findings, EDB affects the spermatid development without directly affecting spermatozoa: the resulting decrease in fertility appears after a time of latency and is severe, although slowly reversible. The LOEL for transient sterility is 10 mg/kg b.w. po for 10 days in the rat; 2 mg/kg b.w. po for 12 days to bulls are sufficient to induce an increase of abnormally shaped sperms. Some epidemiological studies were performed also in humans exposed in the workplace, confirming the concerns arising from animal experiments. For instance, a marked impairment of semen quality was shown in papaya workers following a 5-year exposure: the geometrical mean of breathing zone exposure was 88 ppb.

Carbon tetrachloride (CCl_4) did not induce reproductive effects in the rat at levels in the diet a high as 200 ppm for 2 years. Moreover, newborn rats appear less sensitive to CCl_4 hepatotoxicity than 7-day-old rats, suggesting that adverse effects are not enhanced in the immature organism [4].

Placental transfer in humans has been demonstrated for *trichloroethene*; the fetal: maternal blood concentration ratio is about 1, with large individual variability. In guinea pigs the concentration in ovaries is about half that in fat and twice than other tissues. Exposure of pregnant mice and rats to 1600 mg/m^3 by inhalation on GD 6-15, 7 hours/day, led to some fetotoxicity in mice. However, due to the small size of the experimental groups (12 litters) and the possible presence of traces of epichlorydrin (see below), this study provides only some indications in order to identify developmental hazards [12].

Vinyl chloride has been tested in several prenatal toxicity studies by inhalation during the organogenesis period: although an increase of minor skeletal changes was seen in mice exposed to 500 ppm, no effects were seen in the rat or in the rabbit at levels as hig as 6000 or 2500 ppm respectively. Exposure to this chemical was associated to fetal loss and malformations in humans, but this hypothesis has not received confirmation [4].

Epichlorohydrin, a solvent, is poorly water-soluble. This compound is an *in vitro* mutagen and a possible carcinogen: it is not teratogenic in the mouse and the rat after oral exposure and in the rat and the rabbit after inhalatory exposure at dose levels inducing maternal toxicity (80 mg/kg b.w. and 35 mg/m^3, respectively).

However, epichlorohydrin has a significant potential for reproductive toxicity in rodents. No effects have been observed in rabbits exposed for 10 weeks by inhalation concentrations as high as 133 mg/m^3, and sterility has not been reported in humans after occupational exposure. On the other hand reversible male sterility was observed in rats after 5 day-exposure to 15 mg/kg b.w., and after 10-week inhalation or oral exposure (NOEL 19 mg/m^3 and 2 mg/kg b.w., respectively): the oral 10-week NOEL has been used to derive the 10-day HA by USEPA [4]. The reproductive impairment induced by this compound appears selective for the male: in Long-Evans rats treated po for 21 days with 12.5 mg/kg b.w. the velocity and motility of sperms were decreased, while no effects were seen on female parameters at a dose as high as 100 mg/kg b.w. for 14 days [13].

Chlorobenzene, ortho-dichlorobenzene and *para-dichlorobenzene* [4] did not induce adverse effects on conceptuses after inhalation exposure of pregnant rats or rabbits to levels as high as 590 ppm, 400 ppm and 500 ppm, respectively. Long-Evans rats were exposed by inhalation to chlorobenzene in a 2-generation study: an increase in tubular degeneration of testes was observed in F0 males at 450 ppm, and in F1 males at 150 and 450 ppm: the affected males were less efficient in mating, but the average performance of the exposed groups was unimpaired. Systemic toxicity was concurrently induced by the treatment as renal histological lesions were observed at the same exposure levels and increased liver weight was present even at the lowest exposure, i.e., 50 ppm [14]. No long-term reproductive studies are available on the other benzene compounds. According to the WHO [3] the odour thresholds for chlorobenzenes are of a much lower order of magnitude than the toxicologically based limits.

Low levels of freons have been detected in drinking water in northern Italy [15]. The possible reproductive and teratogenic effects of *HCFC 22* have been carefully investigated [16]. No effects on reproductive performance and hormonal levels were observed in male SD rats exposed by inhalation to 175 g/m^3, 5 hours/day for 8 weeks. While no effects, besides slight maternal toxicity, were seen in NZW rabbits exposed by inhalation to 175 g/m^3 (highest dose tested), in the rat there was some limited evidence of a low risk for eye malformations. After further investigation using large experimental groups, this finding was confirmed only at the highest exposure (175 g/m^3). *HCFC 21* generally shows a much higher toxic potency than *HCFC 22*. The few data on reproductive effects which are available, concern inhalation exposure in the rat: the compound impaired implantation (42.7 g/m^3 on GD 6-15) and decreased the nucleic acid content in the ovaries and placenta (0.153 g/m^3 during pregnancy): the biological significance of such finding is unclear. According to the WHO, the health risks from HCFC should be considered taking into account the environmental exposure, which is likely to be low [16]. In particular, *HCFC 22* is not expected to cause problems at environmental exposure levels.

Mention must be made of the highly lipophylic molecule, *2,3,7,8 tetrachlorodibenzo-p-dioxin* (TCDD) due to its great potential for developmental toxicity, which has been investigated in several experimental studies [4]. In the mouse conceptuses, TCDD concentrations on GD 9-10 (yolk sac period) were greater than on GD 11-18 (placental period), pointing out a possibly higher risk for the early phases of organogenesis. In fact TCDD is one of the most potent teratogens known in mice and in rats (NOEL 0.01 μg/kg/day): effects include urinary tract malformations, fetal death, growth retardation, intestinal hemorrhages, fetal edema, and also cleft palate in the mouse, the occurrence of which is related to presence of the Ah receptor. TCDD is also a possible inducer of microsomal enzymes *in utero* and/or, more likely, through secretion in the mother's milk. As for its potential for reproductive impairment, in a 3-generation oral study in the rat modest effects (reduction of gestation index, decreased fetal weight with increased liver relative weight and increased prevalence of dilated pelvis) were observed at a dose level as low as 1 ng/kg b.w./day, which was used by USEPA to define the longer-term HA. The rhesus monkey might be more sensitive than the rat, as an evident decrease in fertility was observed after exposure to 50 ppt in the diet, i.e., 1.5 ng/kg b.w., for 20 months. At present, the epidemiological investigations performed in humans have provided no convincing evidence of an association with spontaneous abortions or malformations, even after the Seveso accident [17].

Finally, an oral intake ≥ 0.07-0.1 mg/kg b.w./day of *polychlorinated biphenils* has been associated in humans with increased miscarriage, low birth weight and a distinct fetal toxic syndrome. Moreover, PCB are substantially trasferred to infants through the milk, and chronic exposure by consumption of fish living in polluted water may be associated with low birthweight and reduced head size in newborns. The health risks from these compounds appear to derive mostly from their bioaccumulation: the very low levels expected to be present in drinking water are likely to play a minor role [18].

Other organic pollutants

A few compounds used as solvents and also present in fuels will be considered here. Transplacental transfer of *styrene* occurs in humans, concentrations in the fetal tissues being somewhat higher than in maternal blood. City resident women have styrene residues in their milk. However, no developmental effects were seen in Wistar rats treated po on GD 6-15 with 180 mg/kg b.w. (a dose level inducing maternal toxicity) [19].

There is some evidence that at high doses (≥ 900 mg/kg po) *toluene* is embryolethal and teratogenic in the mice. Inhalatory exposure (265 ppm, 8 hours/day, on GD 1-21) of pregnant rats resulted in delayed fetal ossification, without signs of maternal toxicity: this finding might cause some concern as this exposure level is near to the chronic NOEL in the rat [4].

With regard to *xylenes*, CFY rats exposed by inhalation on GD 9-14 to 1000 mg/m^3 of mixed xylenes 24 hours/day showed disturbances of ossification (i.e., increased prevalence of extra ribs and fused sternebrae) without concurrent maternal toxicity: however, the taste and odour threshold for xylenes in water are 0.3 and 1.0 mg/l, respectively [4].

Ethylbenzene did not induce adverse effects on conceptuses after inhalatory exposure of pregnant rats or rabbits to levels as high as 1000 ppm. No long-term reproductive studies are available [4]. The deicing agent *ethylene glycol* [4] increased postimplantation loss and induced fetal growth retardation and a distinct pattern of cranio-axial malformations in rats and mice exposed po to high doses during the organogenesis. In the rat the prenatal LOEL and NOEL were the same as for maternal toxicity (1250 and 1000 mg/kg b.w., respectively), while in the mouse the conceptus appeared more sensitive than the adult (LOEL 750 mg/kg b.w. and NOEL not determined *vs* 1500 and 750 mg/kg b.w., respectively).

Herbicides and pesticides

Herbicides have been shown to pose potential problems of water pollution in Italy [20]. Several compounds belonging to such heterogenous group of molecules have been demonstrated to pose no significant risk for reproduction and/or development.

Alachlor did not induce any effect on reproduction, either in a multigeneration study on CD rats at levels ≤ 30 ppm/diet, and in two segment-II studies on CD rats and Dutch Belted rabbits at oral dose levels as high as 400 and 60 mg/kg b.w., respectively [21].

Atrazine has been tested in the rat for effects on reproduction: only a reduced length of pregnancy was seen at the highest dietary concentration tested (500 ppm) with concomitant signs of general toxicity. The compound was not teratogenic at oral dose levels inducing maternal toxicity in the rat and in the rabbit (5 and 70 mg/kg b.w., respectively) [21].

No developmental or reproductive effects have been observed in several studies on *bentazone* (NOEL: 4000 ppm/diet through pregnancy or 180 ppm/diet for 3 generations in the rat, 375 mg/kg b.w. po on GD 6-18 in the rabbit) [21].

The information available on *metholachlor* and *pendimethalin* show comparable results for the two compounds: they did not cause any significant prenatal effect at dose levels inducing maternal toxicity (i.e., 360 and 120 mg/kg b.w. for metolachlor; 500 and 30 mg/kg b.w. for pendimethalin, respectively, in rats and rabbits). In multigeneration studies on rats, the only effect observed at the highest exposure tested (1000 ppm/food) for both compounds was a lesser weight gain in the offspring [21].

According to the results of segment-II tests, *simazine* may exert a significant maternal toxicity without causing observable effects in the litters: maternal LOEL were 30 and 75 mg/kg b.w., respectively, in rats and rabbits. No reproductive impairment was induced by a dietary exposure to simazine as high as 100 ppm in a 3-generation study in the rat [21].

Trifluralin elicited developmental effects (resorptions, fetal retardation) in Wistar rats and Dutch Belted rabbits only at dose levels inducing also evident maternal toxicity (LOEL and NOEL in rats: 100 and 20 mg/kg b.w. po on GD 6-15, respectively) [21].

A few herbicides were shown to have a potential to affect reproduction although only at exposures much higher than those expected to occur in drinking water.

In a 3-generation study with *MCPA* in the rat the preweaning weight gain of F1 and F2 was reduced (LOEL and NOEL: 22.5 and 7.5 mg/kg b.w./day, respectively); fetal growth retardation was observed in a segment-II study in the mouse (LOEL and NOEL: 100 and 25 mg/kg b.w./day). MCPA is not teratogenic in rabbits at oral dose levels as high as 75 mg/kg b.w., while segment-II studies in the rats did yield different results: the compound showed marked developmental toxicity with concurrent maternal toxicity at dietary levels ≥ 1000 ppm, but no effects in a more recent study at dose levels as high as 125 mg/kg b.w. po. MCPA caused an evident teratogenic effect after a single oral administration of 700 mg/kg b.w. to rats on GD 9 or 10 [21].

Pyridate caused no effects in a segment-II test in NZW rabbits at dose levels ≤ 30 mg/kg b.w. po, while in SD rats severe effects were seen both in dams and conceptuses with LOEL and NOEL 300 and 100 mg/kg, respectively. In Wistar rats exposed through the diet, reduced preweaning weight gain was seen at 2500 ppm and increased renal weight at 2500 and 400 ppm, the NOEL was 80 ppm; in such multigeneration studies it is of course difficult to discriminate between general toxicity and possible indicators of developmental effects [21].

According to the Regional Office for Europe of the WHO [22], the most sensitive parameter of *molinate* toxicity is impairment of the reproductive performance in the male rat. The effect is reversible on withdrawal of the exposure, and it has not been confirmed in studies on rabbits and monkeys. The epidemiological data on workers involved in molinate production did not show an effect on fertility. The NOEL for reproductive effects is 0.2 mg/kg b.w. and it has been selected as a basis to derive the human ADI by using a safety factor of 100.

The nematocide *dibromochloropropane* (DBCP) deserves a mention, as its possible effects on reproduction have been studied in the population of Fresno County, California [23, 24]. This compound was shown to cause severe loss of spermatogenesis in humans following occupational exposure. The epidemiological studies on Fresno county people revealed no significant associations between DBCP levels in drinking water and such parameters as birth rates, birth defects, low birthweight and sex ratio in newborns: results were adjusted for age, parity, race and percent of people with Hispanic descent. According to the authors, these results were not surprising: even though skin contact through washing, bathing etc., might increase by a factor of three the intake due to ingestion, the exposure levels deriving from drinking water remained much lower than those caused by workplace exposure.

The fungicide *hexachlorobenzene* (HCB) is a widespread pollutant with a remarkable potential for bioaccumulation. Conflicting evidence exists about HCB teratogenicity: cleft palate was induced by 100 mg/kg b.w. po on GD 7-16 in the CD-1 mouse, while no effects were seen in the Wistar rat at 120 mg/kg b.w. po on GD 6-21. However, in general HCB has a low acute toxicity, but a wide range of biological effects following moderate exposure; it crosses the placenta into the fetal tissues and its presence in milk might pose a hazard for the neonate. In a 4-generation study in the rat, at 80 ppm/diet the pups had elevated liver weight, but recovered if nursed by untreated dams; at 320 ppm and above high mortality was observed in suckling F1 (NOEL 20 ppm). In a 2-generation study in the SD rats, a significant increase in pup mortality was observed. with LOEL of 40 ppm/diet and NOEL of 8 ppm (i.e., 0.4 mg/kg b.w.). Signs of toxicity and deaths were observed in rhesus monkey infants nursed by mothers treated po with 64 mg/kg b.w. for 60 dd. Exposing female minks at doses as low as 1 ppm/diet during gestation and lactation resulted in increased mortality of kits. Following a pollution accident in Turkey, a 95% neonatal mortality was seen within a year among breast-fed infants from mothers exposed through the diet to HCB [3, 4]. Health risks from the presence of HCB in drinking water could exist; however its very poor water solubility has also to be taken into account (0.005 mg/l at 25 °C).

Ethylene thiourea (ETU) [25] is a highly water soluble environmental metabolite of ethylene bisdithiocarbamate fungicides. ETU induces major developmental effects in the rat when administered during organogenesis, i.e., malformations (mostly hydrocephalus, but also microphtalmia and hydronephrosis) and high postnatal lethality. The NOEL for morphological abnormalities is 5 mg/kg b.w. po; however, reduced cranial ossification is still observed at this dose level. In the rabbit developmental effects (increased prenatal lethality and decreased brain weight, without concurrent maternal toxicity) are seen only at much higher oral exposures: NOEL and LOEL are 40 and 80 mg/kg b.w., respectively. No significant developmental toxicity has been observed in the mouse, possibly due to differences in ETU toxicokinetics. Although the teratogenic effects of ETU appear severe and specific, other effects occur at lower exposures and have been utilized to establish a Drinking Water Guideline, i.e., tumorigenic and goitrogenic effects. In particular, thyroid hyperplasia is observed in the rat following a chronic exposure at doses as low as 0.3 mg/kg b.w.: it is not known whether a specific effect on the developing thyroid might occur.

Inorganic contaminants

The effects of *potassium cyanide* on development were investigated in the rat and in the pig following administration in the diet during gestation and lactation. In the rat, no effects were observed at exposures as high as 500 ppm (50 mg/kg b.w.). Decreased relative weights of thyroid, heart and spleen were seen in piglets (LOEL 276.6 ppm); however, histological alterations were observed in the kidneys and thyroid in the dams even at the lowest exposure level (30.3 ppm) [19].

As reported by Funari *et al.* [20], *nitrates/nitrites* represent a low risk for the average individual; however, a large number of individuals are exposed to these pollutants, which may pose a significant concern for infants. The experimental data on reproductive/developmental toxicity have been reviewed by Fan *et al.* [26]: there was no evidence of teratogenic effects, and adverse effects on reproduction were observed at doses about 10^3 the estimated human intake. When 3 g/l sodium nitrite in drinking water were administered to Long Evans rats during pregnancy and lactation, severe anemia, decreased hematopoiesis and liver toxicity were observed in the pups by the 2nd week post-partum; 1 g/l induced hematological effects without significantly altering growth or increasing mortality (NOEL 0.5 g/l). Cross-fostering indicated that exposure during lactation was more critical than exposure *in utero* [27]. Sodium nitrite crosses the placenta in the rat and caused fetotoxicity at a dose level (200 mg/kg b.w.) which increased the maternal methaemoglobin (MetHb) levels; 16.7 mg/kg b.w. po to mice on GD 0-14, 0-16 or 0-18 stimulaed liver erythropoiesis in fetuses as the only appreciable effect [19]. In humans, there is a sufficient knowledge about the effects on infants, allowing a risk assessment [26, 28]. Babies under 3 months of age have higher MetHb levels as compared to the general population ($\leq 3\%$ vs. $\leq 2\%$) and their haemoglobin is also more susceptible to MetHb formation. Moreover, their stomach pH is higher, particularly in bottle-fed infants: bacteria capable of reducing nitrates to nitrites may thus proliferate. For these reasons the drinking water USEPA HA recognizes the bottle fed newborns as the population

group at the greatest risk and provides acceptable intake values calculated for a 4-kg infant in this case only. Methemoglobinemia in infants is the parameter used to derive guideline values; one should also keep in mind that drinking water is the only non-endogenous source of nitrates for infants [19]. In fact, only in <3-month infants are reported cases of clinical or subclinical methemoglobinemia associated with a relatively low nitrate intake, even though only when there is a concurrent exposure to microbial contamination.

The effects of *Fluorides* in humans have been reviewed in [29]. The F concentration in the fetal skeleton and in the teeth increases with the fetal age and with the F concentration in the drinking water used by the mother: however, no significant fetus-mother gradient exists, at higher exposure levels the placenta may even act as a partial barrier. The concentration in human milk is comparable to that in the plasma, thus milk does not contribute significantly to the general exposure. Marked skeletal fluorosis may occur in children, as well as in adults, with high F exposure in drinking water. Skeletal fluorosis may appear at 3-6 mg/l F: while the pathogenesis of the skeletal lesions is the same in children as in adults, the clinical progression may be different and the developing skeleton may be more sensitive than the mature one. Dental fluorosis appears as the most sensitive endpoint. It is a disturbance affecting enamel before the eruption of the tooth: ameloblasts, enamel maturation and mineralization are all likely targets. "Moderate" dental fluorosis (all enamel surface affected and increased wear) appears at ≥ 1.5 mg/l.

Finally, it is worth noting that also *selenium* might enhance cariogenesis in the developing tooth. Monkeys fed a cariogenic diet and exposed to 2 ppm of sodium selenite in drinking water for 15 months and to 1 ppm for 45 months afterwards, showed a selenium-derived effect when compared to controls exposed to the cariogenic diet only. Teeth were not affected after eruption, only during development. Epidemiological studies in humans were not reported [12].

Metals

In rats, oral exposure through drinking water to *cadmium* ($CdCl_3$) before and during gestation induced embryolethality and fetal growth retardation: delayed ossification was the most sensitive parameter. Prenatal LOEL and NOEL were 2.5 and 1.0 mg/kg b.w.: however, these values are well below the human oral NOEL for hemesis (0.043 mg/kg). The body burden in the newborn is small as the placenta is a fairly efficient barrier against Cd, which typically will slowly accumulate with age: therefore, the actual risk for prenatal development deriving from Cd appears limited [13].

Discharge of industrial effluents containing *nickel* compounds may eventually lead to drinking water contamination: the expected human exposure is very unlikely to exceed 20 µg/l [12]. A significant placental transfer of Ni occurs and fetal levels are comparable to those detected in the adults, either in animals and in humans. According to the results of one multigeneration study in the rat, some effects on fetal weight and neonatal mortality are present even at 5 ppm in drinking water (0.43 mg/kg b.w.). However, owing to possible flaws, this experiment was not used to derive the oral HA [19].

According to the WHO [3, 12], children and infants, fetuses *in utero* and pregnant women are the groups most sensitive to environmental exposure to *lead*. Although limited information is available, it has been estimated that children aged 5-10 years ingest about 90 µg/l/day; no reasonable estimates can be made for younger babies, however, the contribution of drinking water to the total Pb intake can be greater. A concentration of 10 µg/100 ml in drinking water will result in an increase in blood Pb of about 4 and 5 µg/100 ml in children and pregnant women, respectively: a significant number of children may thus exceed the recommended Pb blood level, i.e., 30 µg/100 ml. A concentration of 500 µg/100 ml in drinking water will contribute 30% of the oral Pb intake in infants as compared to 25-50% in adults; as for *absorbed* Pb it will contribute 30% in infants, due to higher bioavailability as compared to 20-30% in adults [3]. As reported in a recent review [30], until recently the interference with the elaboration and function of haemoproteins was considered to be the critical endpoint for Pb, with a threshold around 300-400 µg/100 ml blood. However, now the functional integrity of CNS seems susceptible of being compromised at significantly lower levels, particularly in the fetus and young child. Early postnatal neurobehavioral development may be affected at maternal or cord Pb 100 µg/100 ml and even somewhat less, i.e., a level not uncommon in the general population. Cross-sectional studies indicated that postnatal exposure resulting in ≥ 250 µg/100 ml blood may also be associated with deficits in the neurobehavioural development. The actual long-term consequences have yet to be assessed and the basic mechanisms remain unknown: such developmental impairments might be a sensitive manifestation of a more general effect on cellular differentiation and proliferation, linked to the recognized affinity of Pb for aminoacids containing sulphydril groups and/or to interferences with the activity of mitochondria. Many studies on behavioral effects in children dealt with general environmental exposure: in a few instances there was a slight association between adverse effects and Pb concentration in drinking water [12].

Mercury acetate [19] administered to Syrian golden hamsters on GD 8 caused dose-related embryotoxicity, even at 4 mg/kg b.w. (lowest dose tested): this might cause concern as the 60-week NOEL in the rat is 3 mg/kg b.w. However, inorganic Hg shows a poor oral absorption and, according to the WHO, contamination is

unlikely to exceed 0.03 mg/l even in polluted water [3]. *Methylmercury* [31] is a well-known developmental toxicant that will not be dealt with in the present paper, as exposure occurs mostly through fish consumption. At present, reproductive risks cannot be evaluated for some metals, e.g., *barium* or *chromium* [19]. The expected levels of barium or *beryllum* contamination are so low, and beryllium in particular is poorly bioavailable, that no guidelines values were recommended by the WHO [3, 12].

Interactions between chemicals

A one-generation inhalation study was performed in the rat with 10 mg/m^3 of *hydrogen sulphide* + CS_2 (concentration of each compound not given). Remarkable effects were observed in the offspring, especially urogenital and skeletal alterations [32]. However, the taste and odour thresholds for H_2S are 0.05 and 0.1 mg/l, respectively: it is thus unlikely that an individual will consume a harmful amount [19].

A *chemical mixture of 25 chemicals*, performed to model groundwater contamination around hazardous waste sites, was administered in drinking water for 90 days to B6C3F$_1$ mice to assess effects on spermatogenesis [33]. The highest concentration of the mixture (10%) caused an increase in kidney relative weight and a decrease in water consumption: the only effect in reproductive organs was an increased amount of PAS-positive material in caput epididymis lumina, indicating an enhanced secretory activity. According to data not reported in detail, the mixture did not cause alterations of fertility in a continuous breeding study. The authors noted that the intakes of individual molecules which might pose hazards were all very low (e.g., dichloroethane ≥ 4.08 and Hg 0.064 mg/kg b.w. respectively). Also, chemicals in the mixtures were likely to behave according to different patterns: some cumulative compounds (Cd, PCB) were not allowed to reach steady state in 90 days, while others (e.g., benzene) were rapidly absorbed and eliminated.

Conclusions

In the present review the data on reproductive toxicity have been considered, when feasible, by means of the usual approach for non-genotoxic chemicals, i.e., ADI approach (NOEL + safety factor). However it is worth mentioning the "benchmark dose" approach, which has been proposed as an alternative [34].

The benchmark dose is defined as the statistical lower confidence limits to a dose producing some predetermined increase response rate. According to [34], such approach could make a more appropriate use of dose-response curves and permit the utilization of well-performed studies which do not identify a NOEL.

The examination of the data on reproductive effects for the different groups examined allows to identify some chemicals that can represent a hazard:

Chemicals used in drinking water disinfection. - In experimental studies using levels well above those really present (≥ 10 mg/l) sodium chlorite had some adverse effects on reproduction which might not be readily detectable (e.g., decreased fecundity and/or birthweight).

Chlorination by-products. - Further research is required on chloroacetic acid and dichloro- and trichloro acetonitrile, since data derived from screening studies identify a potential for adverse effects on development which should be investigated more closely.

Other halogenated compounds. - Several such compounds (ethylene dibromide, epichlorohydrin) show an almost selective male reproductive toxicity, with a potential for inducing severe, even though reversible, sterility. However, according to the available data, these chemicals pose a risk mainly at occupational exposure conditions. Further research is required on tetrachloroethylene as some potential hazards have been identified, but no risk assessment is presently possible. Finally, such chemicals as TCDD or, among pesticides, HCB are poorly water-soluble and tend to bioaccumulate. On the other hand, they should be considered among the possible water pollutants posing health concerns, owing to their remarkable potentials for reproductive toxicity.

Other organic pollutants. - Data are neither abundant nor do they indicate severe risks for reproduction: styrene or toluene might have an impact on reproduction, but at exposure levels well above those expected to occur in drinking water.

Herbicides. - Most compounds have been shown not to affect reproduction, while a few (MCPA, pyridate) may have an impact at high exposure levels. In particular, molinate and dibromochloropropane deserve attention because of their potential for inducing reversible male sterility.

ETU shows a broad spectrum of toxic effects, including a significant potential for affecting development: however, tumorigenic and goitrogenic effects occur at lower exposure levels.

Inorganic contaminants. - The higher toxicity of nitrates/nitrites in infants and the effects of fluorides on teeth and bone development might make them the chemicals more likely to actually affect babies and children. A concentration of 10 and 1 mg/l of nitrates and nitrites, respectively, should be regarded as safe for the most sensitive group (infants < 3 months) [19]. Initial effects on the enamel can be seen in infants at levels of intake of fluorine as low as 0.2 mg/kg b.w. (≥ 1.5 mg/l in drinking water) [29]. Also with regard to teeth development, the effects of selenium might deserve further investigation.

Metals. - According to the risk assessment data, lead may be considered as one of the widespread pollutants more likely to exert subtle effects on nervous development. More research may be desirable on the long-term effects on reproduction of nickel.

Interaction between chemicals. - Too few data to allow any conclusion.

With few exceptions there is little evidence of reproductive risks associated with exposure to the low concentrations of chemicals normally found in drinking water. However unusual exposure levels and/or patterns may occur during such events as chemical spills (for a review of health effects induced by exposure to waste disposal sites see [35]). In addition, the database on reproductive toxicity for many relevant chemicals is insufficient to enable proper risk assessment to be carried out. Finally, since contaminants are often found as complex mixtures, it is important to determine the mechanism of action of xenobiotics so that predictions about possible interactions can be made.

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