

THE TOXICITY OF LINDANE IN THE FEMALE REPRODUCTIVE SYSTEM: A REVIEW ON THE ARAL SEA

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ABSTRACT

The disaster of the Aral Sea is one of the biggest environmental problem for the central Asia. The extinction of the Aral Sea began in the 60's, as a consequence of the excessive water consumption for cotton fields irrigation. Pesticides, as the γ -hexachlorohexane (HCH) or Lindane, were used to increase cotton yields. After sea shrinkage, the infertile soil contaminated with pesticide residues dispersed salts and toxicants in the atmosphere. Due to intense winds, toxic salty dust poisoned the population around the Aral Sea, with severe health problems. Lindane, recently classified as carcinogenic to humans, showed endocrine disrupting activity. Unfavorable outcomes on pregnancy and birth seems to be due by alterations in meiotic spindle formation, polar body extrusion, embryonic development. This article revises the grave situation of the Aral Sea region on the human's and animal's health, with regard to the effects of Lindane exposure on female reproduction and fertility.

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1. Introduction

The Aral region is situated in the Central Asia, on the border of Kazakhstan and Uzbekistan, to the right from the Caspian Sea. Kazakhstan is in the southern part of the Aral region and covers Kyzylorda, South Kazakhstan and partially Aktyubinsk and Karaganda regions. The area occupies 350 thousand square meters, with a population over 2,7 million people.

People living in the Aral region are facing with one of the most dramatic man-made environmental disaster - connected to intensive agriculture, extensive irrigation and massive pesticide consumption - exerting harmful effects on health, including reproductive failure. In the 60's, the Aral Sea was the fourth sea in the world by size, after the Caspian Sea, the Lake Superior and the Lake Victoria. It had a surface area of 66.500 km², a volume of 970 km³, a maximum depth of 67 m and a total of dissolved salt concentration of 10-11 g/l. Because of the massive water consumption for cotton irrigation, in the 80's the Aral Sea shrunk, to significantly reduce its surface up to 53%, and its volume up to 70% (1,2).

The progressive desiccation of the Aral Sea was associated to an increased

salinity and to the runoff of pesticides and fertilizers, extensively used in response to the decreased water supply to the Aral Sea and to the increased demand for cotton during the Soviet era. Consequently, toxic salts from the new emerged saline desert called Aralkum, were dispersed in dust and salt storms, to further increase levels of pesticides and fertilizers throughout the Aral Sea region and over (3-5).

Airdust dispersion was favored by meteorological characteristics of the Aral region, that is a windy area of the Central Asia characterized by intense and protracted winds, with a maximal speed of 20-25 m/sec. Satellite images indicated that the dust rain may spread to a maximal distance up to 620-700 km, moving a volume of toxic salts and dust corresponding to 100-130 million tons (6,7).

The consequent unfavorable environment led to serious complications on health, particularly due to: 1) poor quality of drinking water, as evidenced by a rapid deterioration of the reservoir water quality, with an increased salinization and water pollution, 2) air pollution, spread even at big distances from dust storm and 3) soil contamination (7-9). Contaminants, from water, air and soil, transferred into plants, animal tissues and humans through the food chain. As a consequence, maternal and infant morbidity and mortality are significantly higher in areas bordering to the Aral Sea,

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as Karakalpakstan and Kzylorda, than in other parts of Uzbekistan and Kazakhstan (10-12).

2. Chemical composition of the major pollutants found in the Aral region.

Pesticides and fertilizers responsible for the Aral Sea pollution include organochlorine, organophosphates and dithiocarbamates. Persistent organochlorine pesticides (OCPs), as dichlorodiphenyltrichloroethane (DDT), dichlorodiphenyldichloroethylene (DDE), hexachlorocyclohexanes (HCHs), are still found in large proportions in soil, underground waters and water of Syrdarya (2). Human exposure to OCPs starts during prenatal life, due to high lipid solubility, neutral character, limited protein binding, and rather small molecular size. OCPs pass the placenta barrier easily by means of passive diffusion, as demonstrated by their presence in the maternal and cord plasma and placenta (13, 14, 15), even if their presence in the placenta seems not to induce any significant primary damage to DNA in terms of DNA strand breaks and changes in the primary chemical structure (16). Their presence was found in women's breast milk, as shown in biomonitoring studies of mothers from Kazakhstan sampled in 1994 and 2005/2006, indicating OCP concentrations of 0.3 and 0.23 mg/Kg lipid, respectively (17, 18). The most predominant pollutants found in maternal and cord blood, as in human milk, from the near Karakalpakstan are the OCP residues of Lindane (α -HCH and β -HCH, probably byproducts of the industrial production of Lindane), DDT (*pp*-DDE), and the most toxic dioxin, 2,3,7,8-TCDD. Therefore, fetuses are exposed to maternal concentrations during pregnancy (10, 17).

The most representative OCPs are the hexachlorocyclohexanes. Hexachlorocyclohexanes contains eight isomers, all stable organic pollutants, possible human carcinogens and endocrine disruptors with proven teratogenic, mutagenic and genotoxic effects (19). The most prominent isomers are the α -, β -, γ -(Lindane), and δ -isomers, all found in concentration ten times or higher in Kazakhstan, respect to background levels of Europe (200 ng/g fat) (17).

The isomer γ -HCH (or Lindane) is a persistent OCP, widely used as insecticide on forage crops and cereals, by application to plants or soil, and as a seed dressing, alone or in combination with fungicides. Lindane and DDT were applied to cotton fields in the Aral region between 1980 and 1992 at a rate of 58 Kg/hectare in Uzbekistan and 78 Kg/hectare in Karakalpakstan, a concentration more than 30 times higher than those used in Russia and USA (20). Moreover, Lindane is widely used topically in lotions, creams or shampoos from the 1950s for head lice, scabies and tick prevention in humans and animals, with adverse reactions reported (21).

Lindane is extremely dangerous for humans and harmful for the environment. Exposure to Lindane may occur from eating contaminated food or by breathing contaminated air during formulation or use. High levels of Lindane and others OCPs were found in foods with elevated lipid content such as sheep and chicken fat, eggs, and cottonseed oil. Given that these foods are widely consumed in the Karakalpak diet, these results are of great concern for the human health (22).

After having entered the food chain, Lindane is readily absorbed from gastrointestinal tract and then distributed to various tissues or organs

accumulating above all in adipose tissues, liver, ovaries and brain. The compound is eliminated only in small amounts in the urine and faeces. Lindane, as other OCPs, can also pass into breastmilk, thus exerting harmful effects on nursing babies (10, 17).

3. The influence of Lindane on female reproduction and fertility.

Increasing evidences on toxicity made recently the Lindane to be classified as "carcinogenic to humans" (Group 1) by the International Agency for Research on Cancer (IARC) based on the statistically significant increases in non-Hodgkin lymphoma risk with increasing occupational exposure (23 and references therein).

The reproductive failure is affected by environmental toxicants, as we already reported for dithiocarbamates, whose endocrine-disrupting activity can induce several disturbances on estrus cycle, thus inducing infertility (24-29). Similarly, high levels of α -, β - and γ -HCH were found in women with recurring abortions (30). Unfavorable outcomes on pregnancy and birth were also described. The born of small for gestational age (SGA) infants was, in fact, correlated to high concentration of Lindane and other HCH isomers, as found in the maternal and cord blood (31). Chronic bioaccumulation in not occupationally and/or environmentally exposed women represents a prenatal exposure hazard on foetuses, as demonstrated by the presence of higher concentrations of Lindane in the maternal blood and placental tissue of intra uterine growth retardation and preterm births cases, respect to term delivery (32, 33).

Endometriosis is also considered an estrogen-dependent disease. In a population-based study, an increased risk of endometriosis has been related to increased serum of β -HCH. β -HCH is a biologically persistent unintentional by-product of γ -HCH production. Results suggested that exposure from extensive past use of environmentally persistent OCPs may affect the health of the current generation of reproductive-age women with hormonally mediated diseases (34).

Increased concentrations of pesticides in the blood of females living in cotton-growing regions of the South Kazakhstan was recently associated with delayed physical and sexual development, relatively late puberty, and reduced level of gonadotropic hormones, estradiol and IGF1 (35).

Reproductive toxicity was demonstrated also in animals. In fact, pregnancy rates, estradiol and LH levels were affected by Lindane in ewes (36), while in pregnant mice the pesticide caused the absence of implantation site, fetuses resorption and pups death (37).

Lindane may act as endocrine disruptors through the disturbance of normal estrogen-progesterone ratio, thus influencing reproductive outcomes. *In vivo* studies on mice treated on gestational days 9–16 with 15 mg/kg bw/day of Lindane showed subtle effects in F1 pups on the female reproductive development as increase in uterus weight, earlier vaginal patency and reduced diameters of primary oocytes at fully sexual maturity, likely mediated by the ER β pathway (38). Data from *in vitro* studies highlighted the action of Lindane in affecting cell viability/proliferation at a concentration $>1\mu\text{M}$ via ER β , thus speculating that Lindane could interfere with cell cycle entry decision interfering with ER β , particularly in reproductive tissues (36).

When Lindane was given to pregnant mice through the digestive system during the early period of germ cell formation, it caused a marked

reduction of the female and male germ cells within the developing gonads (38). The ability of isolated primordial germ cells to grow in culture was reduced also by a brief preincubation of Lindane, perhaps via apoptotic cell death as seen by increase in caspase-3 activity and a decrease in the phosphorylation of AKT kinase (39, 40).

Germinal vesicle breakdown (GVBD) was not inhibited by Lindane in mouse oocytes, even when used at 100 μM ; however, formation of the first meiotic spindle and extrusion of the first polar body were altered (41). At a concentration of 29.0 mg/mL the insecticide resulted in more than 50% degeneration of bovine cumulus-oocyte-complexes (COCs) (42). Moreover, fertilization and cleavage after *in vitro* maturation in the presence of 7.25 mg/mL were not reduced in comparison to unexposed controls but the subsequent embryonic development was affected (42). Differently, Lindane exposure on developing mouse oocytes *in vivo* led to an increase of irreversible damage, as lysis and fragmentation, in two-cell mouse embryos but not in early cleavage embryos (43).

The reproductive toxicity of Lindane can be a consequence of the inhibition of gap junction formation between the oocyte and surrounding granulosa cells (44). Lindane is, in fact, a general gap junction blocker, specifically acting on Cx43 in granulosa cells (45).

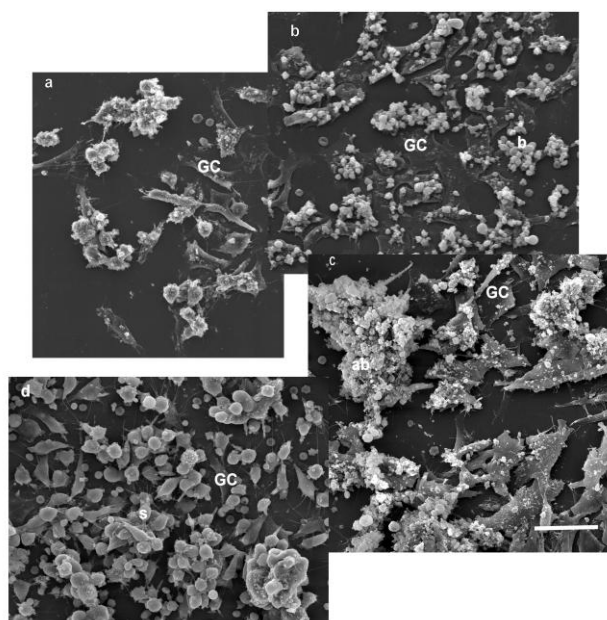


Figure 1 - Representative low magnification micrographs showing the morphological aspect of mice granulosa cells cultured *in vitro* for 48 hours without (a, control) or with increasing concentration of Lindane (b: 1 μM ; c: 10 μM ; d: 100 μM).

From a healthy aspect of polymorphic granulosa cells, richly covered by microvilli and pseudopodia (a), dose-dependent detrimental effects of Lindane are seen: blebbing (b), microvilli reduction with the appearance of a smoother aspect (c), extensive presence of apoptotic bodies and degenerated cells (d). GC: granulosa cell; b: bleb; s: smooth granulosa cells; ab: apoptotic bodies. Scanning Electron Microscopy, bar: 40 μm .

In agreement with the above data, our ultrastructural observations by scanning electron microscopy (SEM) (see refs. 46-50 for methodology) on mice granulosa cells cultured with increasing concentrations of Lindane (1-100 μM), showed a reduction of intercellular communications and a morphological pattern of presumptive apoptosis, evidenced by dose-dependent reduction of microvilli, surface smoothing, blebbing, presence of apoptotic bodies and cell degeneration (Figure 1).

This ultrastructural pattern, associated to a dose-dependent increase of p53, made us to hypothesize the Lindane-induced apoptosis of granulosa cells *in vitro* (51).

4. Conclusions

The above reported data evidenced the deleterious effects of environmental toxicants on the female reproduction *in utero*, in the neonatal and prepubertal periods and adulthood (52, 53). Toxicant exposure, by reducing the fertility, will cause a growing recourse to assisted reproductive techniques as *in vitro* maturation and cryopreservation, with connected side-effects (54-56).

Latest data on Lindane-induced harmful effects made recently the upgrade by the International Agency for Research on Cancer (IARC) from “probable carcinogenic” to “carcinogenic to humans” (22). Due to the action of OCPs including Lindane, in the promotion of tumor angiogenesis via estrogen receptor alpha (52, 53), further studies assessing possible morphological alterations of the angiogenic patterns, as we previously did (58-60), are encouraged.

Evidences here reported should emphasize the need for common strategies to preserve women’s reproductive health, especially for emerging countries, like those in the Aral region, where environmental or occupational exposure to pesticides, often in combination to other toxicants, can induce severe detrimental effects on human’s health.

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