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Trichlorfon exposure, spindle aberrations and nondisjunction in mammalian oocytes

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Abstract. Consumption of trichlorfon-poisoned fish by women in a small Hungarian village has been associated with trisomy resulting from an error of meiosis II in oogenesis. We therefore examined mouse oocytes exposed for 3 h during fertilization to 50 μg/ml trichlorfon. Spindle morphology was not visibly altered by the pesticide. Chromosomes segregated normally at anaphase II with no induction of aneuploidy. However, formation of a spindle was disturbed in many oocytes resuming meiosis I in the presence of trichlorfon. In spite of the spindle aberrations and the failure of bivalents to align properly at the equator, oocytes did not become meiotically arrested but progressed to metaphase II. At this stage, spindles were highly abnormal, and chromosomes were often totally unaligned, unattached or dispersed on the elongated and disorganized spindle. By causing spindle aberrations and influencing chromosome congression, trichlorfon appears, therefore, to predispose mammalian oocytes to random chromosome segregation, especially when they undergo a first division and develop to metaphase II during exposure. This is the first case in which environmentally induced human trisomy can be correlated with spindle aberrations induced by chemical exposure. Our observations suggest that oocytes may not possess a checkpoint sensing displacement of chromosomes from the equator at meiosis I and may therefore be prone to nondisjunction.

Introduction

Trisomy is still one of the major causes of reduced fertility, congenital abnormalities, fetal loss, reduced life span, and mental retardation in the human. One etiological factor identified so far is advanced maternal age (reviewed by Griffin 1996; Hassold et al. 1996; Eichenlaub-Ritter 1996, 1998). It is unclear whether and to what extent exposure to chemical and environmental factors can advance aging effects, modulate the rate of nondisjunction in female meiosis or cause locally and temporally restricted increases in the rate of spontaneous abortion, congenital malformation or occurrence of trisomic conceptuses. From retrospective, molecular analysis of extra chromosomes in human trisomies, it appears that most errors in chromosome segregation arise in maternal meiosis I (for review see Eichenlaub-Ritter 1996, 1998). Study of the segregation of polymorphic markers shows that even those trisomies appear to be initiated during meiosis I, which can be attributed to nondisjunction of chromatids at anaphase II (Lamb et al. 1996). Chiasmata near the centromere may generally have a high risk of remaining unresolved for a prolonged time during meiosis I of oogenesis (Koehler et al. 1996) and chromosomes with excess pericentromeric recombination may therefore be at risk of segregating reductionally at anaphase II. Data on trisomy imply that certain other chromosomal configurations are primarily susceptible to nondisjunction in meiosis. In females, although not all trisomy cases follow this rule (e.g. Bugge et al. 1998). In contrast, studies in rodents suggest that both meiosis I and II may be equally sensitive to the aneugenic activities of chemicals (e.g. Mailhes et al. 1994, 1997; Zuelke and Perreault 1995; Marchetti et al. 1996; Jeffay et al. 1996). It is not known whether some chemicals may preferentially induce an error at first or meiosis II.

One case, in which there was direct evidence for a causal relationship between an inadvertent environmental exposure and trisomy in the human was reported by Czeizel et al. (1993). Consumption of fish poisoned by the organophosphate insecticide trichlorfon by women of a small Hungarian village at around the time of conception was correlated with a locally and temporally restricted, unusually high rate of miscarriage, congenital malformation and children with Downs syndrome in the following period (Czeizel et al. 1993). Retrospective molecular analysis of the origin of the extra chromosome in the informative trisomy 21 cases suggested that an error at meiosis II in females had given rise to the condition. There-

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fore, trichlorfon was suspected as the responsible teratogen and meiotic aneugen causing the errors in chromosome segregation at anaphase II of oogenesis.

Trichlorfon is commonly used as a broad-spectrum pesticide, e.g., under the trade name Diptrex. Owing to its comparatively mild side effects and good anthelmintic properties it has also been used in human therapy, for instance in the treatment of schistosomiasis since the 1960's (e.g. Talaat et al. 1963). The same chemical, termed metrifonate, has also found clinical use more recently in the treatment of Alzheimer patients (Cuttler et al. 1998). Trichlorfon (or metrifonate) is nonenzymatically converted and dehydrochlorinated to dichlorvos, an irreversible organophosphate choline esterase inhibitor (Hinz et al. 1996). However, trichlorfon and dichlorvos may also induce promutagenic methylation damage (e.g., Budawi 1998). The mutagenic activity of trichlorfon was shown in the dominant-lethal test in rodents (Dedek et al. 1975). Dichlorvos induced sister chromatid exchanges in somatic cells (e.g., Madrigal-Bujaidar et al. 1993) and, at high doses, was teratogenic in rodents (Courtney et al. 1986).

Doherty et al. (1996) showed only recently that exposure of somatic cells to elevated concentrations of trichlorfon (more than 20 μg/ml) at neutral pH, at which it is most efficiently converted to dichlorvos, caused an increase in centromere-negative micronuclei, indicative of chromosome breakage. Unlike this, transient treatment of lymphoblastoid cell lines with trichlorfon at pH 5.5, at which the mother compound is presumably taken up into the cells before conversion to dichlorvos, significantly increased the number of centromere-positive micronuclei in the cytochalasin-binucleate test. Taken together these findings suggest that trichlorfon may be an environmental mitotic aneugen. It appeared therefore of primary importance also to assess the influence of the pesticide on (i) chromosome segregation in anaphase II of mammalian oogenesis and (ii) on spindle formation and meiotic progression of mammalian oocytes during completion of meiosis I.

The mouse egg is a good model system for such tests since it can easily be fertilized in vitro (e.g. Hogan et al. 1986). To facilitate chromosomal analysis and distinguish between an influence of the pesticide on chromosome segregation rather than on early embryonic development, we established a protocol whereby fertilized oocytes could be chromosomally analyzed at anaphase II.

Furthermore, mouse oocytes spontaneously and rather synchronously resume maturation and complete the first meiotic division in vitro after isolation from the follicle. In vitro maturing mouse oocytes are a useful model for the identification of the aneugenic properties of drugs and environmental chemicals or relevant metabolites and sensitive times in meiosis in females (Eichenlaub-Ritter and Betzendahl 1995; Eichenlaub-Ritter et al. 1996; Yin et al. 1998), and for analysis of the mechanisms responsible for age-related and chemically induced nondisjunction in mammalian oogenesis. They are amenable to biochemical and immunological approaches or characterization of the behavior of cell components, such as chromosomes and mitochondria (Yin et al. 1998).

In this study we first analyzed chromosome segregation and spindle morphology of in vitro fertilized mouse oocytes progressing to anaphase II. In a second set of experiments, we characterized spindle formation, chromosome behavior and meiotic progression in mouse oocytes maturing in vitro to metaphase II in the presence of the pesticide. These studies show for the first time that a suspected environmental aneugen implicated in trisomy formation in the human induces severe spindle abnormalities in mammalian oogenesis. Moreover, our observations suggest that oocytes may be uniquely susceptible to chemicals and disturbances influencing chromosome alignment.

Materials and methods

Maturation-competent mouse oocytes were obtained from sexually mature MF1 outbred mice of our colony during hormonally unstimulated cycles (Eichenlaub-Ritter and Betzendahl 1995; Soewarto et al. 1995). Oocytes were isolated from ovaries of mice at diestrous (Soewarto et al. 1995), freed of follicle cells and matured in M2 medium in vitro as previously described (e.g., Eichenlaub-Ritter and Boll 1989; Eichenlaub-Ritter and Betzendahl 1995). Trichlorfon (same batch as previously used in lymphoblastoid cell cultures, Doherty et al. 1996) was kindly provided by Jim Parry (Univ. Swansea, UK), dissolved in water at a concentration of 100 mg/ml and stored in aliquots at –20°C for up to 2 months. For oocyte treatment, it was diluted in M2 medium to a final concentration of 50 μg/ml shortly before use. Typically, 20–30 oocytes were incubated in 1 ml of M2 medium with or without trichlorfon in an ungassed incubator at 36°C under mineral oil for 16 h. At this time most oocytes had emitted a first polar body and were arrested in metaphase II.

Processing for anti-tubulin immunofluorescence was done at 8 h or 16 h of culture according to our standard procedure (e.g., Eichenlaub-Ritter et al. 1988; Eichenlaub-Ritter and Betzendahl 1995; Soewarto et al. 1995). In short, the zona was removed enzymatically with pronase, oocytes were extracted in a microtubule-stabilizing solution containing detergent at 36°C, attached to slides and briefly fixed in methanol at –20°C. After rehydration in PBS, they were labeled with antibody. Alternately, to retain the polar body and observe the three-dimensional morphology of the entire zona-intact oocyte, oocytes from culture were fixed in a fibrin clot according to the method described by Hunt et al. (1995). After formaldehyde fixation and blocking, slides were incubated with the first antibody (a monoclonal anti-α-tubulin, Sigma), washed with PBS and reacted with the second antibody (fluorescein isothiocyanate-labeled rabbit anti-mouse antibody, Sigma) (Soewarto et al. 1995). Chromosomes were stained by 4', 6-diamidino-2-phenylindole or propidium iodide according to standard procedures, slides mounted with DABCO (diaminobicyclooctane; Sigma) as antifade and viewed with conventional fluorescence optics and the appropriate filters or with a laser scanning microscope as previously described (Eichenlaub-Ritter and Betzendahl 1995). Immunofluorescent images were recorded on film or by CCD camera and laser scanning microscopy images were later combined to yield images of the entire oocyte, the morphology of the oocyte spindle and the relative positioning of chromosomes on it (Eichenlaub-Ritter and Betzendahl 1995; Eichenlaub-Ritter et al. 1996).

For analysis of chemically induced aberrations in meiosis II, sexually mature MF1 mice were superovulated with 8 IU pregnant mare serum, followed 48 h later by 8 IU hCG (human chorionic gonadotrophin). 12.5 h later, oocytes were isolated from ovulated oocyte-cumulus complexes from the ampullae and placed into pregassed Toyoda medium with 10% fetal calf serum (Toyoda et al. 1989). Epididymal sperm was obtained from male mice of the CBA strain, capacitated for 1 h in Toyoda medium and added to the oocytes at a concentration of 1–2×10^6sperm/ml. At 13.5 h post

hCG, oocytes were incubated together with sperm and cultured for 2 h when most fertilized eggs had reached anaphase II of the second maturation division. They were then washed in M2 medium and further processed for immunofluorescence or spread for cytogenetic analysis. Some fertilized eggs were incubated further to first mitotic division after transfer to pregassed M16 medium (Hogan et al. 1986) and cultured overnight in the presence of 10^{-5} mM vinblastine sulfate (Sigma). Metaphase-arrested one-cell embryos were then spread for chromosomal analysis.

For drug exposure, oocytes were incubated in Toyoda medium plus 50 µg/ml trichlorfon for 1 h prior to fertilization, then transferred to drug-free medium and fertilized. Alternately, in most experiments trichlorfon was present in the medium 1 h before fertilization plus 2 h during fertilization (in total 3 h) until oocytes were spread for chromosomal analysis or processed for immunofluorescence.

Spreading, hypotonic treatment, fixation and C-banding of oocytes and fertilized eggs was done as previously described (e.g., Eichenlaub-Ritter and Boll 1989; Soewarto et al. 1995).

Results

Influence of trichlorfon on fertilization, and chromosome segregation and spindles at anaphase II

When oocytes are fertilized in vitro, a high percentage become activated and form a second polar body within the following 5 h of culture. In one-cell zygotes cultured overnight in the presence of vinblastin, syngamy and the migration of the male and female pronucleus toward each other is prevented. They arrest at metaphase of the first zygotic division such that the condensed chromosomes of the female and male pronucleus can be observed after spreading (Fig. 1a). Errors in meiosis II leading to numerical aberrations of the female chromosomes can thus be analyzed. However, since it is not possible to distinguish the male from the female chromosomes in each zygote and chromosomes of the male and female pronucleus often intermingle, the yield of cells that can be analyzed is usually limited. Also, the number of embryos reaching first mitosis after trichlorfon treatment appears to be diminished relative to controls (data not shown). Therefore, we developed an alternate method in which chromosome segregation can be examined directly in trichlorfon-exposed, fertilized oocytes at anaphase II. When mouse eggs are spread 2 h after fertilization, most of the fertilized ones have reached late anaphase II and are in the process of emitting a second polar body, but their chromosomes are still condensed (Fig. 1b, b'). The two sets of presumptive oocyte and polar body chromosomes are usually spatially separated from each other such that nondisjunction or errors in segregation of chromatids during anaphase II can be detected (Fig. 1b, b'). In the following this technique was employed to determine aneuploidy in female meiosis II after fertilization (Table 1) in the presence of trichlorfon exposure (Table 2).

Trichlorfon has no significant influence on the rate of fertilization at a concentration of 50 µg/ml (Table 1), which induces centromere-positive micronuclei in the cytochalasin-binucleate cell assay (Doherty et al. 1996). C-banding reveals that the pesticide does not affect the sep-

Table 1. Fertilization of mouse oocytes in absence or presence of 50 µg/ml Trichlorfon

Treatment	Number of oocytes	Fertilized	Unfertilized
Control	385	262 (68.1%)	123 (31.9%)
Trichlorfon 1 h	73	57 (78.1%)*	16 (21.9%)
Trichlorfon 3 h	300	183 (61.0%)*	117 (39.0%)

* No significant difference from control

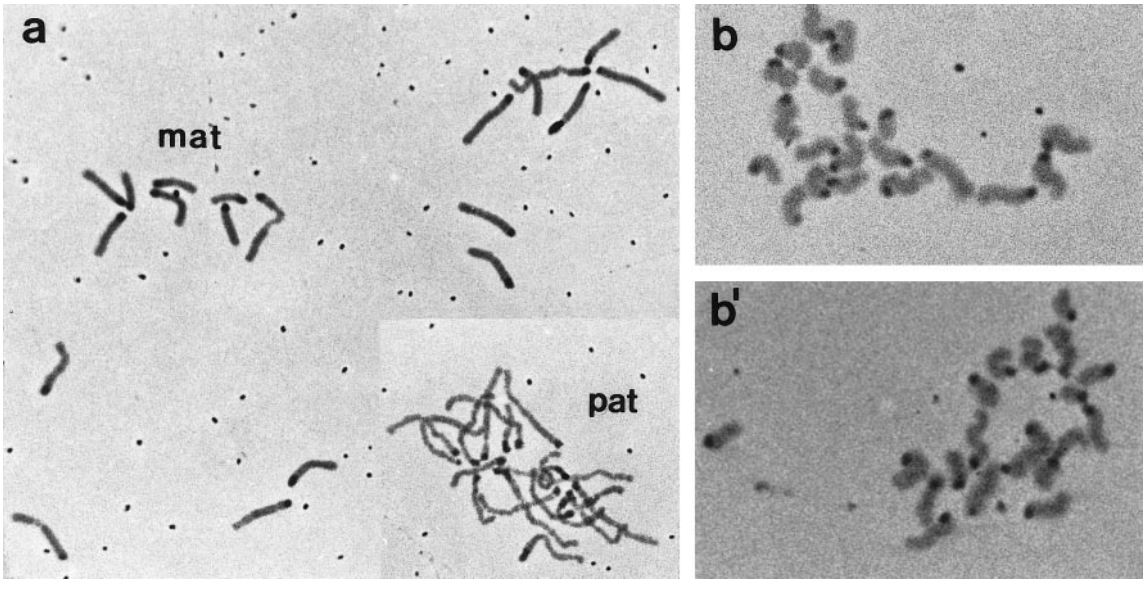

Fig. 1a. 20 maternal (*mat*) and paternal (*pat*, *inset*) chromosomes, respectively, in a spread, C-banded one-cell embryo. **b, b'** Faithful separation of maternal metaphase II chromosomes at anaphase II with two groups of 20 chromatids each, in a trichlorfon-exposed mouse egg 2 h after fertilization (3 h trichlorfon in total)

Table 2. Aneuploidy in fertilized mouse oocytes exposed to 50 µg/ml trichlorfon for 3 h

Treatment	Number of oocytes in anaphase II	Euploid	Hyperploid	Hypoploid
Control	101	90 (89.1%)	1 (1%)	10 (9.9%)
Trichlorfon	81	67 (82.7%)	1 (1.2%)*	13 (16%)

* No significant difference from control

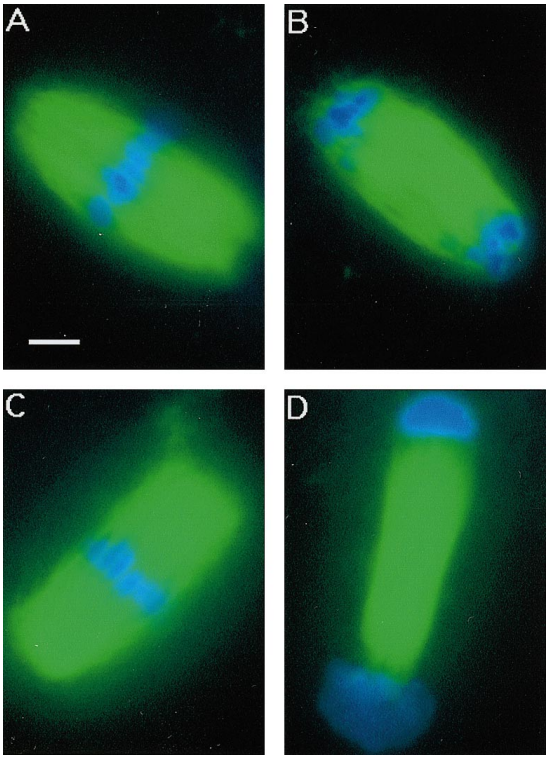

Fig. 2A–D. No obvious differences between spindles of controls (**A, B**) or oocytes exposed for 3 h to 50 µg/ml trichlorfon (**C, D**), failing to become fertilized and still in metaphase II after ageing in vitro for 3 h (**A, C**) or fertilized and in anaphase II (**B, D**). *Bar* represents 5 µm

aration of chromatids and the distribution of chromosomes at anaphase II (Table 2). Spindles of oocytes progressing into anaphase II in the presence of the pesticide (Fig. 2D, Table 3), and spindles of unfertilized oocytes remaining arrested in metaphase II (Fig. 2C) and aged in vitro for 3 h in the presence of the drug do not exhibit any obvious difference to those of untreated controls (Fig. 2A, B). From this we conclude that acute trichlorfon exposure during fertilization and anaphase II progression does not disturb spindle integrity of mammalian oocytes at metaphase II and anaphase II such that errors in chromosome segregation are induced.

Spindles and chromosomes of in vitro maturing oocytes exposed to trichlorfon

Since it appears that oocytes may not be sensitive to trichlorfon once they have reached metaphase II and are ovulated, we suspected that errors in chromosome segregation may be induced by exposure of oocytes to the pesticide during meiosis I. Therefore, we treated mouse oocytes with 50 µg/ml trichlorfon throughout resumption of maturation in vitro.

The majority of mouse oocytes isolated from antral follicles and placed into M2 medium mature to metaphase II during 16 h of culture. They initiate anaphase I and emit a polar body about 9 h after resumption of maturation. Most oocytes of the control possess normal bipolar prometaphase I to metaphase I spindles at 8 h of culture (Fig. 3A, B). Congression of bivalents to the spindle equator is completed in a high percentage of the control oocytes. Oocytes exposed to 50 µg/ml trichlorfon resolve the germinal vesicle and initiate spindle formation at about the same time as controls (data not shown). However, at 8 h of culture spindles are aberrant in a large percentage of oocytes (Fig. 3C, D, Table 4). Characteristically, spindles are bipolar but do not consist of two similarly shaped half spindles. Instead, many spindles are asymmetric with one small and one comparatively broad spindle pole (Fig. 3C, D). In several oocytes the density of microtubules in the central part of the spindle appears to be reduced. In the aberrant spindles as well as the more normally shaped ones bivalents are frequently spread over the entire spindle body (Fig. 3D), and, especially in more advanced stages of meiosis I, single or multiple bivalents appear incapable of aligning properly at the equator.

Since kinetochores lacking microtubule attachment and tension are involved in a metaphase/anaphase checkpoint in mitotic cells of many eukaryotes and in male meiosis of insect spermatocytes (for references see Kallio et al. 1998; Rieder and Salmon 1998, Waters et al. 1998), we expected that the displacement of chromosomes from the spindle equator and the lack of microtubules in the central spindle in many trichlorfon-exposed mouse oocytes may arrest them in meiosis I or that this may delay the progression into anaphase I significantly in a portion of oocytes, similar to what we found in diazepam-exposed oocytes (Yin et al. 1998). However, polar body formation occurs at about the same time in the trichlorfon-exposed as in the control oocytes (data not shown) and at 16 h of culture the majority of oocytes from both experimental groups have reached metaphase II (Table 5).

Table 3. Spindle and chromosome alignment/distribution in anaphase II oocytes exposed for 3 h to 50 µg/ml trichlorfon and controls

Treatment	Number of oocytes	Spindles		Chromosomes	
		Normal	Aberrant	Normal	Aberrant
Control	52	52 (100%)	0 (0%)	52 (100%)	0 (0%)
Trichlorfon	25	23 (92.0%)	2 (8.0%)	25 (100%)	0 (0%)

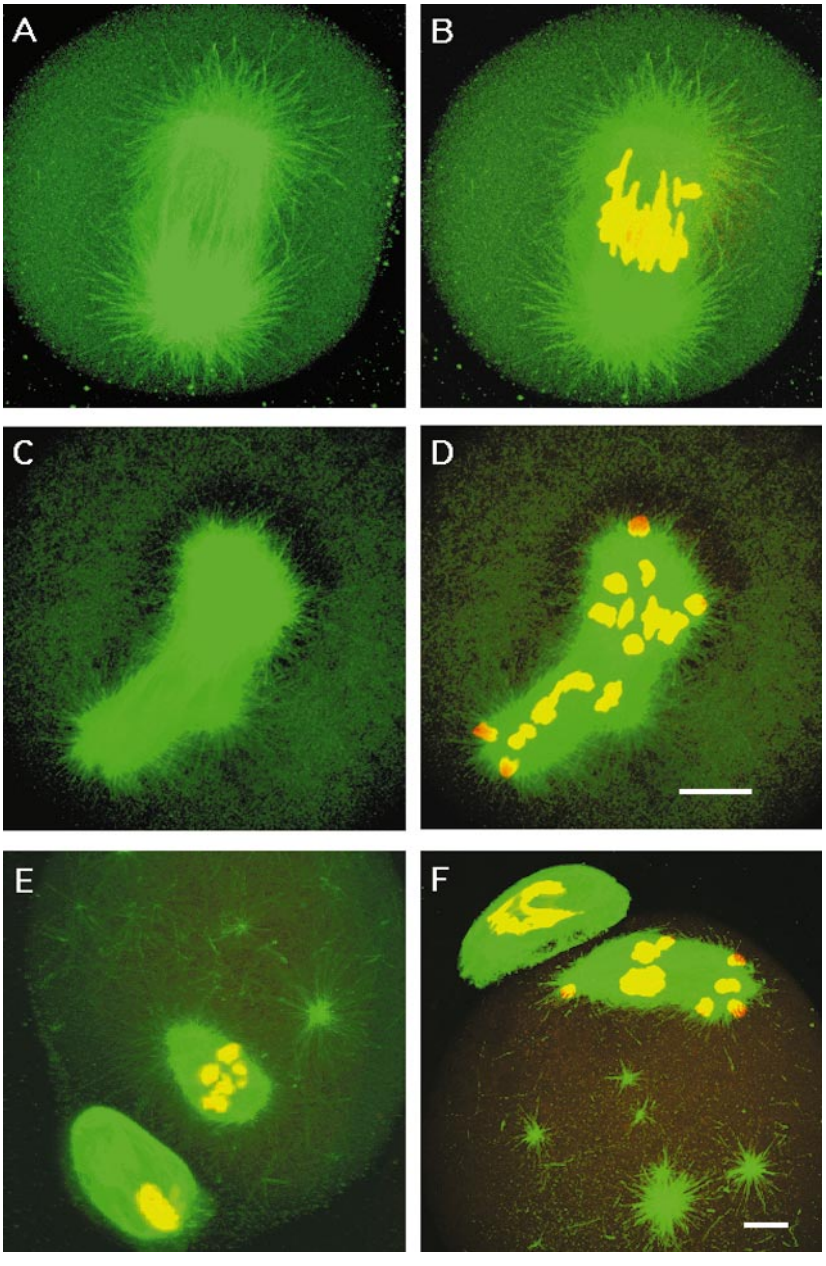

Fig. 3A–F. Confocal laser microscopic images of spindles and chromosomes of control and trichlorfon-exposed oocytes fixed in a fibrin clot after 8 h (**A–D**) and 16 h (**E, F**) of maturation in vitro. **A, B** Typical symmetric, anastral metaphase I spindle (*green*) in control oocyte with bivalents (*yellow*) nearly all assembled at the equator. **C, D** Trichlorfon-treated oocyte with asymmetric meiosis I spindle (*green*) and unaligned chromosomes (*yellow*). **E** View at an angle of about 45° onto the long axis of a metaphase II spindle (*green*, *center*) with chromosomes assembled at the equatorial plane (*yellow*), and on microtubules and chromosomes of the first polar body (*left lower part*) in control oocyte after 16 h of culture. **F** Near normal metaphase II spindle (*green*) but unaligned, dispersed chromosomes in oocyte proper (*center*), and microtubules and chromosomes in first polar body (*left upper corner*) after maturation for 16 h in the presence of trichlorfon. *Bar* in **D** for **A–D**, and in **F** for **E**, **F**, respectively, represents 5 µm

Table 4. Spindles in mouse oocytes matured for 8 h in the presence of 50 µg/ml trichlorfon

Treatment	Number of oocytes	Spindle morphology in meiosis I	
		Normal	Aberrant
Control	56	56 (100%)	0 (0%)
Trichlorfon	87	56 (64.4%)*	31 (35.8%)*

* Significantly different from control, $P<0.001$

Most of the oocytes matured in the presence of the pesticide still formed a bipolar spindle. However, spindles were highly aberrant in a large percentage of the metaphase II stages (Figs. 3F, 4d–f). Thus, several oocytes contained extremely elongated, small spindles with clusters of chromosomes at different positions (Fig. 4e, e'). Others had two astral arrays resembling half spindles barely connected by interdigitating microtubules in the interpolar region (Fig. 4d). Chromosomes were frequently unaligned and located at different distances from the poles (Fig. 4d'). Even those oocytes with a comparatively normal spindle exhibited unaligned chromosomes (Fig. 3F).

The number of oocytes with normal bipolar, anastral spindles and well-aligned metaphase II chromosomes was decreased in the the trichlorfon-exposed oocytes as compared with the controls, showing that the pesticide disturbed spindle formation and chromosome alignment significantly (Table 6).

To conclude, the observations on spindles in meiosis I oocytes, the normal progression of treated oocytes to metaphase II and the highly aberrant morphology of spindles as well as the aberrant behavior of chromosomes in

Table 5. Resumption of maturation in vitro of mouse oocytes exposed to 50 μ g/ml trichlorfon for 16 h

Treatment	Number of oocytes	GV	GVBD	PB	Act.
Control	879	48 (5.4%)	105 (11.7%)	692 (77.2%)	0 (0%)
Trichlorfon	814	60 (7.4%)	81 (10%)	673 (82.7%)	0 (0%)

GV, germinal vesicle; GVBD, germinal vesicle breakdown; PB, polar body; Act., activated

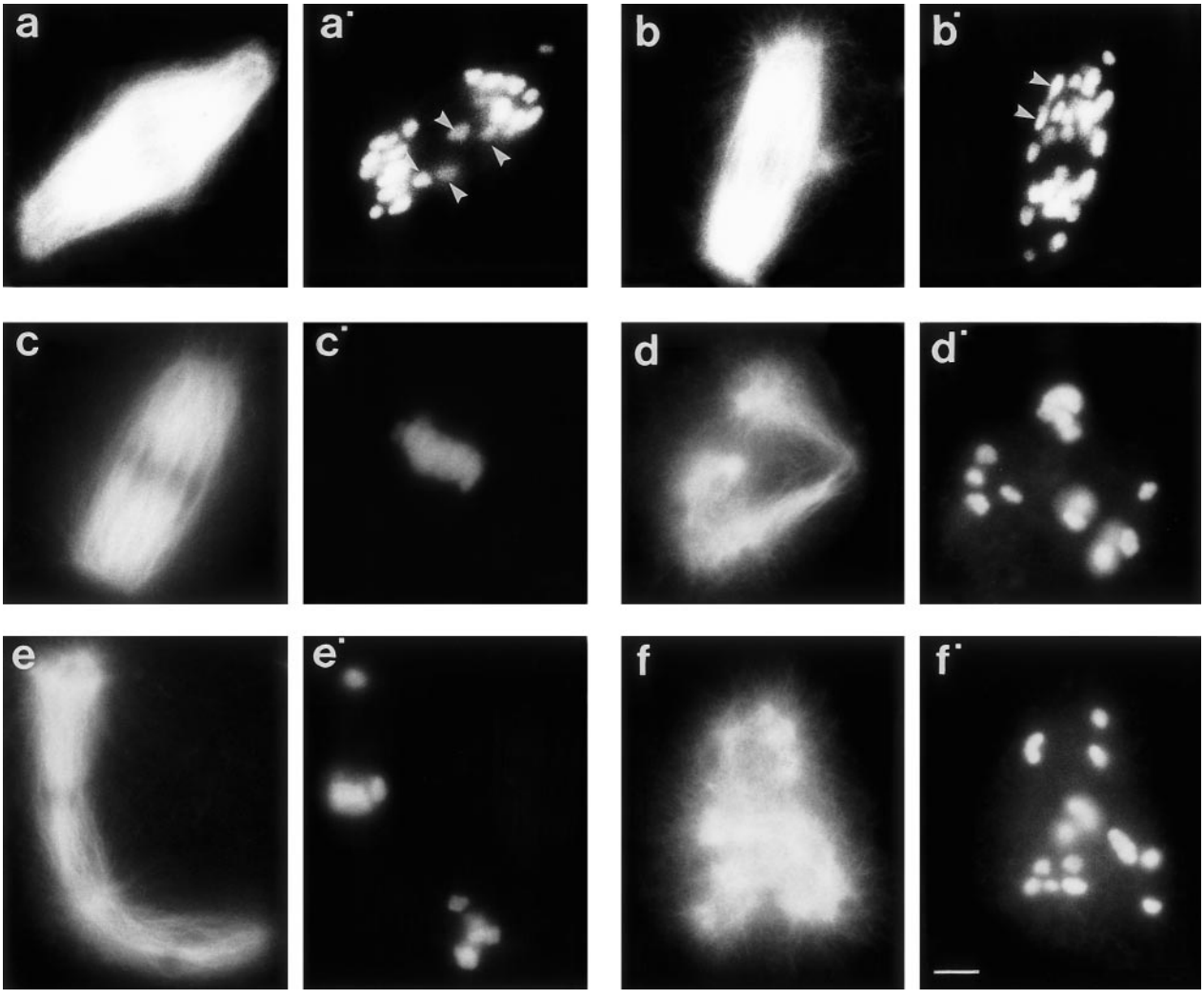

Fig. 4a–f'. Immunofluorescent images of extracted, fixed oocytes with anaphase I (**a, b**) and metaphase II spindles (**c–f**) and 4' 6-diamidino-2-phenylindole-stained chromosomes in oocytes (**a'**,**b'; c'**, **f'**) after 8 h (**a, b**) or 16 h (**c–f**) of maturation in vitro in the absence (**a, c**) or presence (**b, d–f**) of 50 μg/ml trichlorfon. **a'** Although homologs of individual bivalents in controls characteristically separate sequentially they migrate mostly as a front to the poles. Pairs of univalents (*arrowheads*) appear to separate from a position close to the equator. **b'** Univalents of chromosomes in trichlorfon-treated oocytes appear to separate from all locations in the spindle (*arrowheads*) and chromosomes are characteristically spread over the spindle body at anaphase I. **c'** Chromosomes in control metaphase II oocytes are aligned. **d–f'** Spindles in trichlorfon-exposed oocytes are asymmetric and aberrant with unaligned chromosomes. *Bar* represents 5 μm

Table 6. Spindles and chromosome alignment in mouse oocytes matured for 16 h in the presence of 50 μ g/ml trichlorfon

Treatment	Number of oocytes	Spindle morphology in metaphase II		Chromosome alignment	
		Normal	Aberrant	Aligned	Displaced
Control	176	173 (98.3%)	3 (0.8%)	158 (88.8%)	18 (10.2%)
Trichlorfon	157	85 (54.1%)*	72 (45.9%)*	67 (42.7%)*	90 (57.3%)*

* Significantly different from control, $P<0.001$

meiosis II suggest that the pesticide affects spindle formation and chromosome congression to the metaphase plate. Provided the chemically exposed aberrant metaphase II oocytes do not arrest at metaphase II irreversibly but can also progress into anaphase II as in the first maturation division, the pesticide-treated oocytes are at high risk of errors in chromosome segregation, especially during meiosis II, in which spindle disturbances are more severe compared with meiosis I. Therefore, inadvertent exposure of mammalian oocytes to trichlorfon during resumption of maturation may make them prone to nondisjunction and, in consequence, predispose the fertilized egg and embryos to trisomy.

Discussion

Trichlorfon influences spindle formation and chromosome behavior in mammalian oocytes

The coincidence of trisomy due to a maternal second meiosis II error and the consumption of fish poisoned by trichlorfon in a small Hungarian population (Czeizel et al. 1993) led us to suspect that the pesticide is a meiotic aneugen specifically affecting spindles and chromosomes in meiosis II of mammalian oogenesis. However, the present study shows that a brief exposure of oocytes after ovulation to a concentration of the chemical that induces mitotic nondisjunction (Doherty et al. 1996) does not visibly influence the morphology of the meiosis II spindle. Furthermore, once the oocyte has reached metaphase II, the drug does not appear to interfere significantly with fertilization, spindle dynamics and the faithful separation of chromatids at anaphase II.

This is different for meiosis I in which it causes the dispersal and displacement of chromosomes from the spindle equator. This may be mainly due to an interference of the chemical with chromosome congression at prophase. Due to the severe spindle abnormalities and the failures of chromosomes to align properly, pesticide-exposed oocytes appear to have a high risk of errors in chromosome segregation, particularly after metaphase II. Preliminary evidence obtained from cytogenetic analysis supports the notion that trichlorfon is a meiotic aneugen since we found an increase in aneuploid metaphase II oocytes but no evidence for structural aberrations (Cukurcam et al., in preparation). Increased rates of chromosomal loss in peripheral lymphocytes of self-poisoned patients surviving suicide suggest that trichlorfon is also an aneugen in vivo (Czeizel 1994). Alzheimer patients treated with up to 7.5 mg/kg body weight of the drug had a peak level of 7.2±2 μg/ml metrifonate/trichlorfon in their plasma (Unni et al. 1994). Here we show that a seven times higher concentration of the pesticide significantly disturbs spindle formation and chromosome alignment in mouse oocytes.

Metrifonate readily crosses the blood-brain barrier (Taylor 1997), and therefore may also have access to the ovary, the follicular fluid and the oocyte when it becomes inadvertently ingested. Potentially long-term effects of chronic or temporal exposure of oocytes and follicles to the pesticide have still to be characterized to assess the dangers of environmental exposure and long-term medication. Alzheimer patients are usually beyond the reproductive age and trichlorfon treatment may therefore not have consequences for their fertility, but this may be different for other populations exposed to the pesticide.

Mechanism of trichlorfon-induced spindle disturbances

Trichlorfon becomes converted to dichlorvos in vivo (e.g., Hinz et al. 1996) and significantly and irreversibly affects the activity of esterases, e.g., in brain (Pachecka et al. 1977). Other mechanisms of action of the drug have been suggested (Van der Staay et al. 1996) but there is so far no evidence that the pesticide interferes with tubulin polymerization at physiologically relevant concentrations. The alterations in spindle formation and in chromosome behavior found after trichlorfon treatment resemble those induced by the phosphatase inhibitor okadaic acid (OA) (Alexandre et al. 1991; Gavin et al. 1991; de Pennart et al. 1993). OA also interferes with chromosome congression and induces the formation of extremely long, narrow spindles and spindles with few interpolar microtubules (de Pennart et al. 1993). OA alters protein phosphorylation, enhances meiotic resumption and interferes with spindle formation (Rime and Ozon 1990; Alexandre et al. 1991; de Pennart et al. 1993). Trichlorfon and dichlorvos reduce the activity of plasma phosphatases, but do not inhibit phosphatases directly in vitro (Pacheka et al. 1980). Another organophosphate cholinesterase inhibitor, carbaryl, and its metabolite 1-naphthol, appear to influence protein phosphorylation, oxidative phosphorylation and the activity of protein kinases and phosphatases directly or indirectly (Renglin et al. 1998). We found preliminary evidence that the expression of MPM2-reactive phospho-epitopes is altered in trichlorfon-exposed oocytes but further analysis is required.

Dichlorvos stimulates lipid peroxidation and induces oxidative damage in hepatic microsomes in vitro in the presence of NADPH (Yamano and Morita 1992). Spindle disturbances due to oxidative damage have been postulated to be important in the etiology of maternal age-related nondisjunction (Tarin et al. 1998). The initial lesion causing errors in chromosome segregation during female meiosis in response to exposure to chemicals such as trichlorfon may be similar. Our findings suggest that the period of oogenesis preceding ovulation may be the most sensitive for chemically induced disturbances predisposing to chromosomal malsegregation at both anaphase I and II, similar to what was proposed for age-related trisomy formation in the human (Lamb et al. 1996).

Cell cycle progression and checkpoint controls are different between mitotic cells and female meiosis/ oogenesis in mammals

Several drugs, in particular those affecting microtubule polymerization, induce a temporary arrest of maturation

and delay meiotic progression (e.g., Marchetti and Mailhes 1994; Can and Albertini 1997; Yin et al. 1998). This probably involves checkpoints for spindle integrity in the oocyte (e.g., Eichenlaub-Ritter and Boll 1989; Kubiak et al. 1993; Soewarto et al. 1995; Can and Albertini 1997; Winston 1997). Elements of the control of spindle formation, chromosome segregation and cell cycle progression are highly conserved in eukaryotes but there may also be characteristic differences between mitosis, meiosis, species and the sexes (Rieder et al. 1993), and unique features of cell cycle control and expression of meiotic factors in mammalian oogenesis (Fulka et al. 1995a, b, 1997; Duesbery et al. 1997). We found that treatment of mouse oocytes with up to 100 μg/ml trichlorfon does not result in altered meiotic cell cycle kinetics (Cukurcam et al. unpublished) while exposure of somatic cells to 60 μg/ml trichlorfon leads to a mitotic delay (Madrigal-Bujaidar et al. 1993). Moreover, meiotic progression from metaphase I to anaphase I is not delayed in the presence of an unpaired gonosome in female meiosis (Hunt et al. 1995; LeMaire-Adkins et al. 1997). Previously we found that the two X univalents separated in diazepam-exposed mouse oocytes in meiosis I in spite of the presence of other displaced chromosomes (Yin et al. 1998). Therefore, mammalian oocytes may be uniquely suceptible to conditions affecting chromosome congression since they may not become meiotically arrested but progress to anaphase with displaced chromosomes and are therefore at high risk of chromosome malsegregation and nondisjunction.

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