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REPRODUCTIVE EFFECTS OF THE FUNGICIDE THIRAM. EPIDEMIOLOGICAL AND EXPERIMENTAL STUDIES

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ABSTRACT: Aim: To assess the effects of chronical exposure to thiram on the female reproductive function by comparative approach of some epidemiological and experimental aspects. **Material and methods**: Five years epidemiological cohort study on 132 women occupationally exposed to thiram, in the synthesis industry, were compared to matched controls. Exposure evaluation by GC measurements of thiram in workplaces air and biotoxicological tests, too. Fertility questionnaires were used. The experiment studied thiram effects on fertility as well as genotoxic, embriotoxic and teratogenic potential of thiram in chronic experiments on Wistar female rats by using three doses. **Results and discussion**: High concentrations of thiram in the workplaces air, and important levels of its metabolite in urine were found. The epidemiological research has showed among the exposed women a significant decrease of the fertility, a higher frequency of spontaneous abortions (OR=3.52, 95% CI: 2.25-5.50), and a significantly increased risk of congenital malformations (OR=11.57, 95% CI: 2.13-66.86). The mutagenetic investigations indicated a significant increase in the number of chromosomal aberrations (p=0.004) and of micronuclei (p=0.01). In chronic experimental administration, the highest dose (1/3 LD_{50}) determined a significant increase of the tardive resorbtion rate (p=0.004) and also a high proportion of major malformations (p=0.0001); the medium dose (1/9 LD_{50}) and the low dose (1/27 LD_{50}) generated a high rate of foetal loss. These results are confirmed by high rates of structural chromosomal aberrations and also micronucleus high rates. **Conclusions**: Epidemiological and experimental studies support the hypothesis that high exposure to thiram determines a significant fertility decrease and the qualitative impairment of offspring.
Key words: thiram, occupational exposure, experiments, reproductive function

Rezumat. **Scop**: Evaluarea efectelor expunerii cronice la thiram asupra funcţiei reproductive feminine prin abordarea comparativă a unor aspecte epidemiologice şi experimentale. **Material şi metodă**: din punct de vedere metodologic s-a realizat un studiu epidemiologic de cohortă pe 132 femei expuse ocupaţional la thiram în industria de sinteză, comparativ cu un lot martor. Expunerea a fost evaluată prin măsurători GC ale thiramului în aerul locurilor de muncă şi prin teste biotoxicologice. Au fost utilizate chestionare de fertilitate. Experimental s-au studiat efectele thiramului asupra fertilităţii, precum şi potenţialul genotoxic, embriotoxic şi teratogenic al acestei noxe în experimente cronice pe şobolani Wistar femele, folosind trei doze. **Rezultate şi discuţii**: Rezultatele au arătat concentraţii mari de thiram în aerul locurilor de muncă şi nivele importante ale metabolitului său în urină. Studiul epidemiologic a arătat o scădere semnificativă a fertilităţii, o scădere mai mare a avorturilor spontane (OR=3,52; 95% CI: 2,25-5,50), şi un risc semnificativ crescut pentru malformaţii congenitale (OR=11,57; 95%

CI:2,13-66,86). Testele de mutageneză au arătat o creştere semnificativă a numărului aberaţiilor cromosomale (p=0,004) şi a micronucleilor (p=0,01). În administrarea experimentală cronică, doza cea mai mare (1/3 LD_{50}) a determinat o creştere semnificativă a ratei resorbţiilor tardive (p=0,004) precum şi a malformaţiilor majore (p=0,0001); doza medie (1/9 LD_{50}) şi doza scăzută (1/27 LD_{50}) au generat o rată crescută a avorturilor. Aceste rezultate sunt confirmate şi de ratele crescute ale aberaţiilor cromomosomale structurale şi ale micronucleilor. **Concluzii**: studiile epidemiologice şi experimentale sprijină ipoteza că expunerea la nivele mai mari de thiram determină o scădere semnificativă a fertilităţii şi o afectare a calităţii produsului de concepţie.

Cuvinte cheie: thiram, expunere ocupaţională, experimente, funcţia de reproducere

INTRODUCTION

We conceived a study which is comparable with the most recent research mentioned in literature (1-3). The assessment of the potential adverse effects of the dithiocarbamic fungicide thiram on female reproductive function, were performed by comparative approach of some epidemiological and experimental aspects.

1. The epidemiological aspect: to evaluate the possible reproductive outcomes of the dithiocarbamic fungicide thiram in chronic occupational exposure among women working in the synthesis industry of fungicides.
2. The experimental aspect had in view the genotoxic, embriotoxic and teratogenic potential action of dithiocarbamic fungicide thiram by mutagenicity and teratogenicity tests in rats.

MATERIAL AND METHODS

First stage of a five years cohort study on 132 women occupationally exposed to thiram compared to a matched control lot of 122 women working in a garments manufacture in the same geographic area.

The experimental aspect design comprised chronic experiment studies on Wistar rats by using three thiram doses representing 1/3, 1/9 and 1/27 of DL_{50} .

1. Estimation of the occupational risk:

The dithiocarbamic fungicide thiram has been assessed by the gas chromatography (GC) method in the air of the workplaces during the duration of the study (4,5).

The absorption rate of the toxic substance in the organism has been determined by biotoxicological tests: iodin-azide test and natrium diethil-dithiocarbamat. These determinations have also been performed during the study (6,7).

2. The ***investigations of some aspects concerning the fertility*** have been performed in the framework of a complex study regarding the health status in relation to the exposure risk.

We have used complex fertility questionnaires concerning reproductive history, health status, environmental and occupational exposures to noxious agents.

The measured effects have been: the menstruation setting up, time to pregnancy, spontaneous abortions, birth weight, gestation age, congenital malformations, number of the

pregnancies and births, late foetal death, status of health of the children at the birth time.

3. The ***cytogenetic investigations*** included chromosomal aberration and micronuclei test.

4. The fertility effects as well as the embriotoxic and teratogenic potential of dithiocarbamic fungicide thiram have been assessed in ***chronic experiments*** on Wistar rats by administrating (in diet) thiram dispersed in distilled water, in three doses representing: 1/3 of DL_{50} = 250 mg/kg body weight, 1/9 of DL_{50} = 83.3 mg/kg body weight and1/27 of DL_{50} = 27.8 mg/kg body weight

Group I (50 females and 10 males) received 250 mg/Kg body weight.

Group II (50 females and 10 males) received 83.3 mg/Kg body weight.

Group III (50 females and 10 males) received 27.8 mg/kg body weight

The toxic doses were administered once weekly for two months before mating, and in the 9^{th}, 12^{th}, 15^{th} and 18^{th} day of the gestation.

The control group (34 females and 7 males Wistar rats) received only distilled water.

For the teratogenicity test the female rats were put to sleep in the 21^{th} day of gestation.

5. The ***mutagenic effects*** have been assessed in chronic experiment on male Wistar rats by using the same three doses.

RESULTS AND DISCUSSION

1. Estimation of the occupational risk:

The GC analyse of the dithiocarbamic fungicide thiram *in the workplaces air* has shown a multitude of average concentrations variable in time and space, of which common character was a *constant and significant exceeding of the standards* both for Treshold Limit Value - Time Weighted Average (TLV-TWA) and for TLV-Ceiling. Mean values of dithiocarbamic fungicide thiram in the air of the workplaces during the investigated period, *significantly increased during this first stage* (from 35.5±11.2 up to 76.5±28.5), reaching a value almost five times greater than the TLV - TWA.

The *iodine-azide test* and the *natrium diethil-dithiocarbamat urinary elimination* showed significant differences between the end and the beginning of the shift (at 75-80% subjects for the first test and at 86-92% for the second test) thus pointing out *relevant increases of thiram absorption during the activity.*

2. The epidemiological research

The exposed lot had 132 women working in the fungicides synthesis industry and the control lot had 122 women working in garments manufacture.

The exposed lot had a mean age of 22.4 ± 2.5 years and the control, 23.2 ± 2.1 years. The socio-economic status and educational level were comparable. The comparative analysis of some other confounding factors for the fertility assessment indicated no significant differences between exposed and controls: all women are married, the divorce percentage is 1 *vs.* 2; first menses age 12.4 *vs.* 12.1 years, genital infections 11.4% *vs.* 12.5%; 3% *vs.* 5% are smokers; 0% *vs.*

2% have light drinking habits and drug consumption is without importance.

The results showed a significant impairment of the reproduction function:

- ***ovarian dysfunction:*** irregularities of the oestral cycle 52% *vs.* 18%; longer menses (> 5 days) 12% *vs.* 5%; increasing of the time to pregnancy: 30 *vs.* 10 months;
- spontaneous abortions - ***significant difference between the spontaneous abortion rates*** (abortions/ woman) 1.9 at the exposed women *vs.* 0.6 in control (p=0.005);
- ***significant difference between women with spontaneous abortions:*** 59.8% at the exposed vs. 29.5% in controls (Yates'chi square: 22.35, p<0.0001, OR=3.56, 95% CI:2.11-6.0); it is to be noticed the increase of the spontaneous abortions after hiring only for the exposed women as well as the fact that the great majority of abortions happened in the first three months of pregnancy;
- ***sexual disturbances*** pointed by the psychological testing (Psychoratter) 60% *vs.* 28.9%, χ^2 =11.9, p<0.001;
- ***hormone changes*** - abnormal decrease of the urinary levels of 17 keto-steroids at 99% of the exposed *vs.* 12% at the controls.

We have especially found an inverse correlation between these levels and the urinary elimination of DDC-Na: r = 0.62, p<0.001;

- ***Fertility impairment*** at the exposed women *vs.* control:
  - The increase of the women without pregnancies 27% *vs.* 4%, the difference being significant;
  - Significant lower level (p<0.01) of the births number (129 *vs.* 214 at the control)
  - Significant decrease (p<0.001) of the births number after *vs.* before hiring
  - Significant lower level (p<0.01) of the fertility rate at the end of the investigation (1.2 *vs.* 2.2) as well as significant decrease of the fertility rate after vs. before hiring;
  - Significant difference between born and desired children at the exposed lot;
- The study of ***children's health status at the birth*** has showed:
  - The percentage of normal born children was smaller, to the exposed group compared to the control (82.1 *vs.* 97).
  - The malformations have been found in a percentage of 6 for the exposed women compared to 1 in controls; (Yates'chi square: 11.19, p=0.0008, OR = 11.57, 95% CI: 2.13-66.86).
  - The underweight has been found in 18 % of the exposed compared to 5.5 % in controls.
  - The stillborn percentage is higher at the exposed women 3 *vs.* 0.2 in controls.

These results seem to be in agreement with other recent researches (1,8).

3. The cytogenetic investigations after three years of exposure have pointed out a significant increase of the structural chromosomal aberrations (cromatide gaps, cromatide breaks etc.) at 37% of the exposed group compared to 1.64% in the controls (Yates'chi square: 21.48, p<0.000004, OR=16.15, 95% CI: 3.76-69.45) and

micronuclei (8.33% *vs.*0.82%, Yates'chi square: 6.37, p=0.01, OR=11.0, 95% CI: 1.4-86.53). These results are to some extent comparable with other studies (9, 10).

4. Teratogenicity aspects: concerning the prolificity indicators, the number of alive foetuses decreased in the intoxicated groups compared to the controls (5.02 *vs.* 3.58%), and the resorbtions (especially the precocious ones), have significantly increased in the lowest dose group (1/27 LD50 = 27.8 mg/bw) compared to controls, suggesting embriotoxic effects of dithiocarbamic fungicide thiram (table 1).

Table 1. **Fertility and prolificity indicators**

Group	FEMALE RATS			Avg. of alive foetuses on gestant	Total nidations			
	empty	excluded uterus	gestant		Alive foetuses	Dead foetuses	Resorbtions	
							Precocious	Tardive
CONTROL		59			156			
	33	0	26	3.58	93(59.6%)	1(0.6%)	62(39.7%)	
	55.9%	0%	44.1%				56	8
Group I		92			344			
1/3 LD50	25	10	47	5.02	236(68.6%)	2(0.6%)	106(30.8%)	
250mg/ bw	27.2%	10.9%	51.1%				73	33
Group II		86			271			
1/9LD50	42	5	39	4.43	173(63.8%)	4(1.5%)	94(34.7%)	
83.3mg/bw	48.8%	5.8%	45.3%				78	16
Group III		74			257			
1/27LD50	28	11	35	3.22	113(44.0%)	5(1.9%)	139(54.1%)	
27.8mg/bw	37.8%	14.9%	47.3%				130	9
TOTAL		252			872			
EXPOSED	95	26	121	4.31	522(59.9%)	11(1.3%)	339(38.9%)	
	37.7%	10.3%	48.0%				281	58

In chronic experimental administration, the highest dose (1/3 DL50) determined a significant increase of the tardive resorbtion rate (31.13% *vs.* 12.9 %, p=0.004).

These results are confirmed and completed by significant effects on cellular level, as: high rates of structural chromosomal aberrations and also micronuclei high rates.

This type of genotoxic potential was confirmed by the information gained from epidemiological studies we performed at the same time as well by the results of other studies (11,12).

The medium dose (1/9 DL50) and the low dose (1/27 DL50) have had negative effects upon the quality indicators of the offspring: foetal weight, the degree of ossification and the malformation of foetus skeletons (table 2).

Table 2. The Quality Indicators of the offspring

Control	**Number**	**Sex**	**Average body weight** (grams)	**Retarded ossification degree** (average score in a 0 to 5 gravity scale)	**Malformation degree** (average score in a 0 to 5 gravity scale)
Control group	50	M	3.90±0.66	1.62±2.17	2.48±2.60
	43	F	3.93±0.58	1.65±2.50	3.30±3.11
	93	Total	3.92±0.62	1.63±2.32	2.86±2.86
Group I 1/3 LD50 250mg/bw	107	M	4.08±0.63	1.12±1.07	2.50±2.13
	129	F	3.86±0.66	1.51±1.55	2.57±2.38
	236	Total	3.96±0.66	1.33±1.36	2.57±2.27
Group II 1/9 LD50 83.3mg/bw	95	M	3.76±0.99	2.69±3.37**	3.62±3.63*
	78	F	3.68±0.81	2.55±3.34	3.49±3.58
	173	Total	3.72±0.91*	2.63±3.35**	3.56±3.60
Group III 1/27 LD50 27.8mg/bw	52	M	3.85±0.76	1.96±2.82	3.33±3.26
	61	F	3.59±0.58**	2.59±3.06	2.92±3.20
	113	Total	3.71±0.67*	2.30±2.96	3.11±3.22
TOTAL EXPOSED	254	M	3.95±0.82	1.88±2.61	3.09±3.04
	268	F	3.74±0.70	2.06±2.60	2.95±2.97
	522	Total	3.83±0.76	1.97±2.60	3.02±3.00

* $p < 0.05$; ** $p < 0.01$

Thiram did not significantly affect male foetuses bodyweight but the low (1/27LD50) and the medium (1/9 LD50) doses evidently diminished the general development of the female foetuses.

The ossification process was equally affected for the male and female foetuses, especially by the low and medium doses.

Minor skeleton malformations were found especially in male foetuses, in the low and medium dose groups.

So, we can assume that Thiram has embriotoxic, foetotoxic and teratogenic effects.

5. The **genotoxic effects** were demonstrated by the chromosome aberration assay in bone marrow cells in both types of experiments and by sperm abnormal morphology test in the chronic experiment. The low dose did not induce increased frequency of chromosome aberrations in bone marrow cells in both experiments, while the higher dose induced a significant increase of cytogenetic alteration frequencies in bone marrow cells: metaphases, especially in subacute experiment (6.83 ± 2.14 *vs.* 1.8 ± 1.47, $p<0.05$). Some similar findings were published (3,9,13).

In chronical experiment both doses and especially the higher one ($p<0.001$) have determined alterations of the sperm morphology.

CONCLUSIONS

- The women occupationally exposed to high levels of thiram, had significant high levels of absorption as demonstrated by the biotoxicological tests.
- The ***epidemiological study*** has shown, until now, a significant *decrease of the fertility*, a significant *increased risk for congenital malformations*, as well as an *increase of the spontaneous abortion ratio*.
- From the ***teratogenicity point of view***, the thiram has determined: *fertility decrease*, *precocious resorbtions increase*, *embriotoxic* effects and *impairment of the quality* indicators of the *offspring*.
- ***Mutagenicity studies*** have emphasized the *genotoxic effects* of thiram.
- The *results* of the epidemiological and experimental studies are *convergent* and support the hypothesis that high occupational exposure to thiram represents an important reproductive hazard for the women.
- In order to elucidate the action mechanisms, where the neuro-endocrine system seems to be very important, it is necessary to perform a deeper study by widening epidemiological investigations and by tackling new experimental aspects.
- The implications of the reproductive function impose a severe revision of the exposure standards, a coercion to respect these standards, an improvement of the medical prophylactic measures, for the fertile age women.

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