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Ethylenethiourea in air and in urine as an indicator of exposure to ethylenebisdithiocarbamate fungicides

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KURTIO P, SAVOLAINEN K. Ethylenethiourea in air and in urine as an indicator of exposure to ethylenebisdithiocarbamate fungicides. *Scand J Work Environ Health* 1990;16:203-7. Ethylenethiourea (ETU) is a ubiquitous impurity of the ethylenebisdithiocarbamate (EBDC) fungicides widely used in agriculture and forestry. In the present study, ETU was used as a measure of the exposure to EBDC on potato farms and in pine nurseries during the application of EBDC fungicides and the weeding of the sprayed vegetation. Biological and hygienic monitoring was carried out through the analysis of ETU in the breathing zone and the urine of exposed workers. Even if the concentrations of ETU in the ambient air of pine nurseries exceeded those of potato farms, the concentrations of ETU in the urine of potato farmers exceeded those of pine nursery workers. This result may have been due better protective equipment in the pine nurseries. The excretion rate was 6-10 ng/h during the first 60 h after the cessation of exposure, and it diminished thereafter to 0.2 ng/h over a 22-d observation period.

Key terms: biological monitoring, occupational hygiene, pine nurseries, potato farms.

Ethylenebisdithiocarbamates (EBDC) are common agricultural fungicides, and they are also widely used in forestry. Ethylenethiourea (ETU), a degradation product and byproduct of the manufacturing of EBDC, is formed in the presence of moisture and oxygen in EBDC formulations. EBDC are also metabolized to ETU in the mammalian organism (1-3). ETU itself is used as an accelerator in the rubber vulcanization process. It is also used in dyes, pharmaceuticals, synthetic resins, and electroplating baths (3). Even though the acute toxicity of pure EBDC is low (3), the health of workers may be jeopardized due to exposure to ETU (4). Moreover, EBDC and ETU may be readily absorbed via the lungs, the gastrointestinal tract, and the skin (5).

ETU causes thyroid hyperplasia and marked alterations in the levels of thyroid hormones in the serum of humans and experimental animals (6, 7), and it is also known for its teratogenic potency (8). ETU is weakly genotoxic in several test systems (9, 10), but is a clear carcinogen in experimental animals (11). The National Institute for Occupational Safety and Health (NIOSH) in the United States considers ETU a potential human carcinogen (12).

In this study, four groups of workers were examined during and after exposure to EBDC fungicides. Biological and hygienic monitoring of the exposure was carried out through measurements of ETU in the ambient air and in urine of EBDC-exposed workers. The objective of the study was to evaluate the internal dose

of ETU and the potential hazard to the health of workers from exposure to EBDC fungicides or ETU.

Subjects and methods

Subjects and the application of the pesticide

The exposure of two groups of pesticide applicators to EBDC fungicides was studied on 38 potato farms. Group I comprised nine men (average age 40 years, body weight 79 kg, and height 178 cm) studied during the summer of 1986. In group II, there were 29 men (average age 41 years, body weight 83 kg, and height 177 cm), and the study was carried out during the summer of 1987. Exposure to EBDC was also studied in a group of workers from four pine nurseries (group III). It consisted of five men (mean age 33 years, body weight 77 kg, and height 177 cm) who applied pesticide in the summer of 1986. A final group (group IV) consisted of 15 women (mean age 44 years, weight 71 kg, and height 162 cm) who weeded in the pine nurseries in the summer of 1987.

The protection of the pesticide applicators on the potato farms (groups I and II) was usually poor, and they never wore respirators to decrease exposure via inhalation. The pine nursery workers (group III) wore respirators (equipped with a particle filter), rubber boots, leather or rubber gloves, and overalls.

The potato fields were sprayed with a 56 % mancozeb or a 80 % maneb formulation. In the pine nurseries, only a maneb formulation was used. The weeding of the vegetation in the nurseries was carried out two weeks after the treatment of the fields with maneb. There was always an interval of several weeks between subsequent sprayings so that each worker was studied after a single application or exposure period.

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A tractor-pulled pump-operated spray equipped with a 300–500 l tank was used for the pesticide application. Pressure during the spraying was 2–4 kp/cm² (2–4 kp = 20–40 N = 20–40 kg · ms⁻²). The height of the nozzles was about 50 cm above the ground. The mean areas of the sprayed fields were 4 ha (group I) and 14 ha (group II) on the potato farms and 3 ha in the pine nurseries (group III). The average application time was 2 (groups I and III) or 4 (group II) h. The workers in group IV did not carry out any application of EBDC fungicides.

Sampling

Air samples for the analyses of ETU in the breathing zone of the workers and in the cabin of the tractors used for fungicide application were collected on membrane filters (0.8 µm, Millipore) with a portable pump (MSA model S) with a flow of 2–3 l/min. The sampling times were equivalent to the application times. Air samples were collected with a Resiprotor[®] pump (20 l/min) during the weighing and the mixing of the pesticide with water (5–15 min).

Group I collected all urine of 10 subsequent urine samples voided after the cessation of application (average follow-up time was 59 h). Group II collected four 24-h urine samples during the 22-d period (days 1, 8, 15, and 22) after the cessation of EBDC exposure. The pine nursery workers in group III collected all urine from 5–15 urinations after the cessation of exposure (average follow-up time was 44 h). A 24-h urine sample was collected from every nursery weeder in group

IV in a manner similar to that used for group II (days 1, 8, 15, and 22). All the workers also gave a blank urine sample prior to the beginning of the exposure.

Analysis for ethylenethiourea

The analysis of ETU in EBDC fungicides on filters and in the urine samples was carried out with a high-pressure liquid chromatograph (HPLC) as described earlier (13). Briefly, a filter and 2 ml of distilled water were kept in a sonicating water bath and mixed.

The ETU in the urine samples (10 ml) was evaporated to dryness. Thereafter, 2 ml of methanol and 150 mg of silica gel were added. Methanol was evaporated, and the sample was transferred to an aluminum oxide column and eluted with 2 % methanol in dichloromethane. The eluate was evaporated, and the sample was dissolved in 0.5 ml of water. Recovery (mean 50 %) of each separate sample preparation was measured with the use of urine spiked with a known amount of ETU. The spiked sample was prepared and analyzed in the same manner as the other samples.

To determine the amount of ETU impurity in the formulations, we weighed a known amount of EBDC formulation in a 50-l measuring flask and diluted it with distilled water.

The HPLC analysis of ETU was carried out with a Hewlett Packard 1090 liquid chromatograph equipped with a diode array detector. The column was a reverse phase Hypersil 5 ODS[®] (column length 25 cm, internal diameter 4.6 mm, Chrompack). The isocratic elution of 5 % methanol in 0.05 M ammonium acetate in water was used. The detection wavelength was 230 nm. The detection limit for ETU was 100 pg/injection; for ETU on a filter sample and in urine the detection limit was 8 ng/sample and 200 ng/l, respectively.

Analysis of the results

The levels of ETU that were at the detection limit of the method but were too low to be reliably quantitated were considered to be at the detection limit, ie, 8 ng/filter for an air sample and 200 ng/l for a urine sample. The means and standard deviations or the standard errors of the means of the concentrations of ETU in air and in urine were calculated. The slope of the elimination phase of ETU in urine was calculated for the first 60 h and for the whole 22-d follow-up period with the method of least squares.

Results

Potato field applicators

If it is assumed that the concentrations of ETU in the air were stable during the whole work period, the exposure via the lungs (ETU µg/m³ × time × 0.02 m³/min breathing rate) was more than tenfold greater in group II (5.2 µg) than in group I (0.4 µg) (table 1) because of the longer application time. The amounts of

Table 1. Ethylenethiourea (ETU) concentrations in the breathing zone of four groups of workers exposed to ethylenedithiocarbamates.

Exposure group ^a	ETU (µg/m ³)					
	Spraying			Weighing and mixing		
	Number of workers	Mean	SEM	Number of workers	Mean	SEM
I	10	0.14	0.06	10	0.87	0.20
II	13	0.80	0.30	13	0.90	0.20
III	5	0.60	0.39	13	1.81	0.58
IV	14	0.01	0.003			

^a Groups I and II: potato field applicators, group III: pine nursery applicators, and group IV: pine nursery field weeders.

Table 2. Excretion of ethylenethiourea (ETU) in urine during the first 24 h after the cessation of exposure to ethylenedithiocarbamates.

Exposure group ^a	ETU (ng/24 h)	
	Mean	SEM
I	3746	885
II	3368	945
III	2172	1047
IV	498	203

^a Group I and II: potato field applicators, group III: pine nursery applicators, and group IV: pine nursery weeders.

Excretion of ETU

Figure 1. Excretion rate (means and standard deviations of the means) of ethylenethiourea (ETU) in the urine (ng/h) of potato field applicators (circles) (groups I and II) and pine nursery weeders (squares) (group IV) after exposure to ethylenebis-dithiocarbamates during pesticide application (groups I and II) and the weeding of the sprayed vegetation (group IV). The ETU concentrations were at the detection limit in group IV after two weeks of follow-up (two last time points).

Excretion of ETU

Figure 2. Excretion rate of ethylenethiourea (ETU) (means and standard deviations of the means) in the urine (ng/h) of potato field applicators (group I) during 60 h after the cessation of exposure to ethylenebis-dithiocarbamate fungicides. The first time point at 10 h after the cessation of the application was omitted from the analysis because of possible continuous exposure for a few hours after the application and because of the effect of dermal absorption. The regression equation is $y = -6x + 455$, where y is the excretion rate of ETU (ng/h), x is the time (h), and the correlation coefficient squared (r^2) is 0.86.

ETU in the urine of the men in these two groups show, however, that the absorbed amounts of EBDC fungicides metabolized to ETU were equal (table 2). The concentrations of ETU during the weighing and mixing of the pesticide (10 min at a time) was similar for both groups (table 1). Therefore, this work phase may have contributed considerably to the absorbed amount of the pesticide.

The concentrations of ETU in the urine of the potato field applicators were between <0.2 and $23 \mu\text{g/l}$. The decrease in the excretion rate of ETU was the most rapid between 20 and 40 h after the cessation of the exposure (figures 1 and 2). The average excretion rate of ETU was 6 ng/h during the first 60 h after the cessation of the exposure, and it diminished to 0.2 ng/h during the 22-d follow-up period, as indicated by the equations given in table 3. The results suggest a two-phase elimination for ETU groups I and II. Slow dermal penetration may have contributed to this result. The variation in the levels of ETU in the urine was great, and it did not allow for a reliable estimation of the elimination half-time of the urinary excretion of ETU (figures 1 and 2).

Pine nursery applicators

The concentration of ETU in the air during the weighing and mixing of pesticides was the highest in the breathing zone of the workers in group III (table 1). The average concentration of ETU in the breathing zone of these workers equaled the pooled concentration of ETU in the breathing zone of the potato field applicators [groups I and II combined 0.54 (SD 0.17) $\mu\text{g/m}^3$]. The concentrations of ETU in the urine of

Table 3. Rate of the excretion of ethylenethiourea (ETU) in urine. The equation $y = a + bx$ gives the slope for the elimination of ETU for each worker group separately. [y = ETU in urine (ng/h), x = time, a = y -intercept (urinary excretion rate (ng/h) of ETU at the cessation of the exposure), b = slope of the elimination]

Exposure group ^a	Follow-up excretion time (h)	Regression coefficients	
		a	b
I	10–60	455	–6
II	24–528	135	–0.2
III	20–50	632	–10
IV	24–528	20	–0.02

^a Groups I and II: potato field applicators, group III: pine nursery applicators, and group IV: pine nursery weeders.

group III were between <0.2 and $5.6 \mu\text{g/l}$. The excretion rate of ETU was 10 ng/h during the first 50 h after the cessation of the exposure (table 3).

Pine nursery field weeders

The concentration of ETU in the breathing zone of group IV exceeded the limit of detection only in four out of 14 samples. The highest air concentration of ETU was $0.04 \mu\text{g/m}^3$ (table 1). The concentrations of ETU in the 24-h urine samples exceeded the limit of detection in only four of the 60 samples. After two weeks (on day 15) ETU could not be detected in the urine samples. (See tables 2 and 3 and figure 1). Figure 1 indicates the marked difference in exposure to EBDC between the potato field applicators (groups I and II) and the pine nursery weeders. (See also table 3.)

Concentration of ETU in maneb and mancozeb formulations

The concentrations of ETU in formulations containing maneb and mancozeb were 59 and 22 mg/g (5.9 and 2.2 %), respectively.

Discussion

The results from the present study showed that the exposure of all the workers exposed to ETU was clearly below the Finnish exposure limit, which is 0.2 mg/m³ for an 8-h workday with a ceiling value of 0.6 mg/m³ for 15 min (14). In the pine nurseries, the concentrations of ETU in the air were relatively high during the periods of pesticide application. However, the absorbed amounts of ETU and EBDC, as illustrated by the concentrations of ETU in the workers' urine, were smaller than those measured for the potato field applicators. The difference was probably due to the use of better protective equipment and clothes among the pine nursery workers. The significance of ETU impurity in EBDC formulations was probably minor because the amount of ETU in the EBDC formulations was small.

During the weeding of sprayed vegetation the probable route of exposure is via the skin. EBDC do not belong to the systemic fungicides, and they remain on plant surfaces (15). In addition, ETU has been found on plants treated with EBDC (16). However, intact ETU disappears from plants and soil with a half-time of less than one week and much more rapidly than EBDC, the half-time of which is four to eight weeks. The half-time of ETU derived from EBDC has been shown to be less than four weeks (17). Therefore, the exposure of the weeders was low, and probably mainly from EBDC fungicides rather than from intact ETU. In agreement with these observations, ETU was not detected on the clothes or on the skin of the weeders (data not shown). The small amounts of ETU in the breathing zones of the weeders could have been due to ETU that had biodegraded from EBDC in the soil (3, 17).

In this study, the excretion rate of ETU in urine was the highest during the first 60 h after the cessation of exposure. Gradually, the rate of the excretion declined. After 21 d, only small amounts of ETU could be detected in the urine of the workers with the highest exposure. The analysis of the rate of excretion of ETU in urine revealed that, after the initial elimination phase of 60 h, the decrease in the elimination rate of ETU was 0.2 ng/h over the whole follow-up period. This finding illustrates the slow rate of urinary elimination of ETU. The present sampling strategy did not allow for an accurate estimation of the urinary elimination half-time for ETU. In addition, the urinary excretion of ETU continued for a surprisingly long time after the cessation of the exposure to EBDC. The exposure to EBDC and ETU was not necessarily terminated,

however, immediately after the pesticide application. The pesticide was usually stored in an unsheltered storage place where frequently used farming equipment was stored. Thus continuous exposure, mainly via the skin, may have played a role and may possibly explain the present results.

Two different phases could still be demonstrated in the urinary elimination of ETU. However, our estimate of the urinary elimination half-time of ETU of about 32–37 h differs from our earlier observation of the elimination half-time of ETU of about 100 h in another group of workers also exposed to EBDC and ETU (18). This difference may be due to the marked differences in the sampling strategies and the modes of exposure. The present results support the view that EBDC are metabolized to ETU in the human body because the exposure to ETU alone cannot explain the amounts of ETU found in the urine.

After the treatment of rodents with maneb, the amount of ETU excreted in the urine was 10–16 % of the dose (2, 19, 20). The amount of ETU excreted in urine after the administration of ETU was 24–63 % for different animal species (2, 21). Intestinal absorption of EBDC may not always be exactly proportional to the dose. For example, cations naturally occurring in food will decrease the absorption, especially at lower doses of EBDC (22). Thus the results on absorption in rodents do not directly apply to man. The elimination rate of different EBDC is complicated by the fact that mancozeb is more resistant to metabolic degradation than maneb (23). Thus, in agreement with the present results, the excretion of ETU is complex, and a clear correlation between concentrations of EBDC in air and ETU in urine cannot be assumed.

Earlier, EBDC, but not ETU, concentrations in the breathing zone, or on the clothes, of workers have been analyzed to estimate the occupational exposure to EBDC (24–28). The exposure limits for dithiocarbamate fungicides vary between 0.1 and 10 mg/m³ (25, 29, 30).

Because ETU is a suspected human carcinogen, it is crucial to determine exposure to it whenever possible. Because of its biological properties and because of the complexity of the biotransformation of EBDC to ETU, ETU in urine is not, however, an ideal choice for the biological monitoring of exposure to EBDC or ETU. However, our measurements of ETU in air and urine indirectly reflect the amount of exposure to EBDC or ETU and show that even a small exposure to EBDC and ETU may result in measurable concentrations of ETU in urine. These measurable concentrations can then be used as a warning signal for exposure to a potentially dangerous substance. It would also be valuable to control the amounts of ETU impurity in EBDC formulations and to find alternatives for compounds which are transformed to ETU via biodegradation or biotransformation.

The measured amounts of ETU in urine in the present study do not, however, support the view that the

observed levels of exposure to ETU and EBDC would readily jeopardize the health of exposed workers.

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