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Asthmatic symptoms after exposure to ethylenebisdithiocarbamates and other pesticides in the Europit field studies

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We conducted a multicenter prospective study to assess the effects of occupational exposure to ethylenebisdithiocarbamate fungicides and/or other pesticides on self-reported asthma and asthmatic symptoms. This multicenter study was conducted among 248 workers exposed to pesticides and 231 non-exposed workers from five field studies. The five field studies were carried out in The Netherlands, Italy, Finland, and two studies in Bulgaria. Subjects constituting this cohort completed a self-administered questionnaire at baseline (before the start of exposure). Ethylenethiourea in urine was determined to assess exposure to ethylenebisdithiocarbamates. In multivariate analyses adjusted for all potential confounders (age, education, residence, smoking, gender, and field study), we found inverse associations, all not statistically significant, between occupational exposure

to pesticides and asthma diagnosis (OR 0.41; 95% CI 0.15–1.11), complaints of chest tightness (OR 0.60; 95% CI 0.36–1.02), wheeze (OR 0.56; 95% CI 0.32–0.98), asthma attack (OR 0.52; 95% CI 0.12–2.25), and asthma medication (OR 0.79; 95% CI 0.25–2.53). Furthermore, we reported null associations for multivariate analysis using ethylenethiourea as determinant for exposure. Although exposure to pesticides remains a potential health risk, our results do not suggest an association between exposure to ethylenebisdithiocarbamates and/or other pesticides used in our study on asthma and asthmatic symptoms.

Key words: asthma; epidemiology; ethylenebisdithiocarbamates; multicenter study; wheeze

Introduction

Asthma is one of the most frequently reported diseases of the airways. Between 100 and 150 million people worldwide suffer from asthma, and this number is increasing.¹ The prevalence of asthma ranges from 2 to 30%, depending on the country and the definition of asthma used.^{2,3} About 15% of the individuals, between 20 and 69 years old, have complaints of wheeze with dyspnea.⁴

Asthma is a respiratory condition, causing dyspnea, chest tightness, and wheezing.^{2,3,5} Allergic asthma is associated with type I hypersensitivity,

that is the immune system produces specific antibodies (IgE antibodies) in response to otherwise harmless allergens causing above-mentioned symptoms.^{6,7} A wide variety of allergens may initiate a recurrent episode of asthma. The most common ubiquitous allergens are organic derivatives such as dandruff and hairs of animals, mites, pollen, and fungi.⁵ Non-specific responses are elicited in hypersensitive asthmatics to a variety of triggers such as cold air, mist, and respiratory irritants like smoke, perfume, and also by some pesticides.^{5,8}

Occupational pesticide exposure may take place either in industry or in agriculture. Although in agriculture, the main route of absorption is the skin, industry workers are exposed mainly by the respiratory tract.⁹ Differing from industry workers, agricultural workers usually use several pesticides from

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different chemical groups, and the levels of exposure may significantly vary over time. Also, the general population can be exposed to pesticides and residues mainly through dietary intake of food and water. In some cases, pesticides show an immunomodulatory activity in humans mainly slight changes in some immune laboratory parameters.¹⁰ The prognostic significance of these changes is still unclear, but the question whether these changes can evolve into overt disease, one of which is asthma, has to be taken into account. The answer to this question could be provided mainly by epidemiological studies. Due to the large size of the pesticide exposed population and the severity of the possible effects, answering this question is a very urgent task for public and occupational health. So far, only a few studies have investigated the possible relation between exposure to certain pesticides (mostly organophosphates or carbamates) and asthma.

In a cross-sectional study by Sprince, *et al.*¹¹ an increased odds ratio (OR) for symptoms of asthma (wheeze, phlegm, flu-like symptoms) was reported in relation to applying pesticides (in general) on livestock. In a cross-sectional study by Senthilselvan, *et al.*¹² a significant association of application of carbamate insecticides with the prevalence of asthma was found in a population of male farmers. A cohort study by Hoppin, *et al.*¹³ explored the associations between wheeze and pesticide use in the past year. The authors found small associations with exposure to the herbicide paraquat and to some organophosphate insecticides (parathion, malathion, and chlorpyrifos). This study—the only study so far that has investigated the relationship between dithiocarbamates and wheeze (an asthmatic symptom)—reported a small association for thiocarbamate (S-ethyl-dipropylthiocarbamate) use. However, no associations between exposure to other thiocarbamates (such as maneb and ziram) and wheezing were observed.¹³ In a study by Jones, *et al.*⁹ 4% of the case subjects and 6% of the control subjects reported increased asthma symptoms after spraying mixtures of different pesticides. The authors concluded that the results suggest asthma symptoms and lung function to be similar among workers with occupational exposure to pesticides and controls with only community-based exposure.⁹

Thus, previous studies demonstrate that some pesticides might enhance asthmatic symptoms. However, little is known about the contribution of ethylenebisdithiocarbamate fungicides (EBDCs)—widely used in agriculture, mainly in fruits, ornamental plants, and vegetables¹⁴—to asthma etiology and more research is needed.

Because available data are suggesting a possible immunostimulator effect exerted by EBDCs,¹⁵ our hypothesis was that individuals occupationally exposed to EBDCs and/or other pesticides might have an increased risk of asthma and/or asthma-related symptoms than non-exposed subjects.

In this multicenter study, we therefore investigated the effects of exposure to EBDC on asthma and/or asthmatic symptoms assessing self-reported asthma and/or asthmatic symptoms of subjects exposed to EBDCs and/or other pesticides.

Material and methods

The study design and population

We conducted a prospective multicenter study (EUROPIT study). The study population consisted of participants of five field studies carried out in The Netherlands, Italy, Finland, and two studies in Bulgaria. The study sites chosen were based on actual information on exposure according to work-related activities. The facilities available for our study, including financial support, allowed a maximal number of 100 individuals per study, that is 50 exposed and 50 controls. In The Netherlands, 42 workers employed in flower bulb growing industry and 40 non-exposed controls (mostly office employees) were included. The Italian field study comprised 48 vineyard workers and 45 non-exposed controls (derived from the same area and matched on main characteristics as age and sex). In Finland, data were collected from 51 potato farmers and 49 non-exposed biological farmers. The first Bulgarian field study was conducted among 52 employees from a factory that produced mainly zineb (an EBDC) and 50 controls (mostly workers from the same factory, with jobs with no exposure to pesticides). In the second Bulgarian field study, data were collected from 55 greenhouse workers producing cucumbers and tomatoes and 45 controls (greenhouse workers with no exposure and office employees). All these field studies combined resulted in a total study population of 248 exposed workers and 231 non-exposed workers. All exposed participants had a job history of exposure to pesticides, whereas controls had never been occupationally exposed.

All participants were informed about the final result of the study and signed an informed consent.

The questionnaire

All subjects completed a self-administered questionnaire at baseline. The questionnaire has been constructed from other (validated) questionnaires to guarantee the quality. The respiratory questions

were partly constructed from the IUATLD (International Union Against Tuberculosis and Lung Disease) questionnaire.^{16–18} Furthermore, the questionnaire has been translated in the local language and later back-translated in English to check on the correct translation. The questionnaire included questions on demographic factors, presence of disease, vaccinations, use of drugs, alcohol, and smoking. Furthermore, job information was covered by questions on job title, company name, duration of employment and, for exposed subjects, type of pesticide used. Asthma and asthmatic symptoms were investigated by questions like: “Have you ever had asthma?” followed by a second question: “Was the asthma diagnosed by a physician?” Participants were also asked about asthmatic symptoms like wheezing (“Have you ever had an attack of wheezing or whistling in your chest?”), chest tightness (“Does your chest ever feel tight or does your breathing become difficult?”), asthma attack (“Have you had an attack of asthma any time in the last 12 months?”), and asthma medication (“Are you currently taking any medicines, tablets, or inhalers for asthma?”).

Urinary ethylenethiourea as biomarker of ethylenedisithiocarbamate exposure

Because ethylenethiourea (ETU) is a major metabolite of EBDCs,¹⁴ the personal level of this exposure was assessed through the determination of ETU in urine. One spot urine sample was obtained from each subject, roughly 30 days after the beginning of the seasonal application of pesticides. Due to the short half-life of urinary ETU in the human body, sampling was performed within 16 hours after the end of nominal exposure. Samples were kept frozen at -20°C before analysis. ETU was isolated from urine by liquid/liquid extraction using dichloromethane, which reacted to form a bis(*tert*butyldimethylsilyl) derivate and analyzed via gas chromatography-mass spectrometry as previously reported.¹⁹ Detection limit for ETU was $0.5\text{ }\mu\text{g/g}$ creatinine.

Statistical analysis

Due to lack of follow-up data on asthma and asthmatic symptoms, analyses were limited to cross-sectional analysis of the available data of exposed workers compared with non-exposed workers. For ETU, a value corresponding to half of the detection limit was assigned to ETU measurements below analytical detection. As urinary ETU was not normally distributed, its values were log-transformed to get a more normal distribution. For this reason the geo-

metric mean of ETU was used, as a descriptor of ETU distribution.

For exposure definition, we used different strategies: subjects were divided into exposed and non-exposed (dichotomous variable) based on predefined job titles. These were confirmed by the questionnaire. Or urinary ETU as a continuous variable were used in analysis.

Multivariate logistic regression modeling is used for the assessment of associations between variables, considering ever been diagnosed with asthma as depending variable (self-reported at baseline questionnaire). The same analyses were carried out with wheezing, chest tightness, asthma attack, and asthma medication as dependent variable. Results are expressed as OR adjusted for field study, demographic variables (age, education, gender, and residence), and all potential confounders (education, gender, residence, field study, and smoking). All OR are presented with 95% confidence intervals (95% CI).

Furthermore, based on an asthma prevalence of 7.2%,²⁰ this study has a power of 80% to detect an two-fold increased asthma prevalence ($\text{OR} = 2.0$), with a one-sided test, $P: 0.05$.

All statistical tests are two tailed. P -values equal to or less than 0.05, or equal to or less than 0.01, were reported as statistically significant. All data were analyzed using SPSS statistical software package.²¹

Results

The distribution of population characteristics is shown in Table 1. By design, the exposed and controls appeared equally distributed among the five field studies.

Overall, diagnosis of asthma is reported more often by controls than by exposed subjects (Table 1). Also wheezing, chest tightness, asthma attack, and asthma medication are reported more by control subjects than exposed subjects. However, none of these differences between exposed and controls were significant.

The excretion of ETU was overall higher in exposed workers than in controls. Moreover, urinary ETU was detected in controls of the majority of field studies, with an overall geometric mean value of $0.5\text{ }\mu\text{g/g}$ creatinine.

Multivariate analysis adjusted for potential confounders for exposed and controls are presented in Table 2. Significant decreased OR was found only for wheeze for subjects exposed to EBDCs and/or other pesticides compared with non-exposed subjects adjusted for all confounders. All associations

Table 1 Characteristics of exposed and control subjects in percentage with number of subjects between brackets, overall and by field study

	Overall	
	Exposed (N = 231)	Control (N = 248)
Gender		
Male	66.5	62.4
Female	33.5	37.6
Residence		
Urban	37.4	63.8
Rural	62.6	36.2
Smoking		
Ever	56.5	57.6
Never	43.5	42.4
Education		
Low	33.3**	9.7
Middle	50.0	41.4
High	16.7	48.9
Regular sunlight exposure		
Yes	78.8**	66.5
No	21.2	33.5
Ever lived on a farm		
Yes	67.6**	36.3
No	32.4	63.7
Asthma diagnosis		
Yes	3.3	8.4
No	96.7	91.6
Wheeze		
Yes	16.3	24.7
No	83.8	75.3
Chest tightness		
Yes	20.0	30.7
No	80.0	69.3
Asthma attack		
Yes	1.7	4.0
No	98.3	96.0
Asthma medication		
Yes	2.9	4.0
No	97.1	96.0
Average age (years) ^a	42.43	42.23
Wine (number of glasses per month) ^a	8.1	8.08
ETU ^{b,c} (µg/g creatinine)	2.7** (8.1) ^d	0.5 (3.7) ^d

ETU, ethylenethiourea.

^aArithmetic mean values.

^bGeometric mean values.

^cDetection limit set at 0.5 µg/g creatinine.

^dGeometric standard deviation.

*Significant difference between exposed vs controls, $P < 0.05$.

**Significant difference between exposed vs controls, $P < 0.01$.

between asthma and asthma-related symptoms are negatively associated with exposure to EBDCs and/or other pesticides. Other statistically not significant associations are reported for asthma diagnosis chest tightness asthma attack and asthma medication

Table 3 shows the same analysis as in Table 2 but now, using urinary ETU as a continuous marker of exposure, expressed in OR per standard deviation increase. Most associations were not statistically significant. We noted a slightly increased OR for asthma diagnosis and asthma medication, although neither were statistically significant.

Furthermore, we analyzed the data using average ETU levels as indication of exposure, expressed in OR per standard deviation increase (results not presented in table). Most associations are null and not statistically significant.

Discussion

In this study, we assessed asthma and/or asthmatic symptoms among participants of the EUROPIT study conducted in The Netherlands, Italy, Finland, and Bulgaria on workers exposed to EBDCs and/or other pesticides.

Exposure assessment was performed grouping people accordingly with their job titles and use of EBDCs, and also through the measurements of urinary ETU, one of EBDCs' major metabolite. A previous study indicates that ETU represents a good indicator of exposure.²² Based on the increase in urinary ETU in exposed compared with controls, we were able to confirm that occupational exposure to EBDCs took place during most of the field studies. However, due to the short half-life of ETU in humans, it reflects only a recent exposure (roughly the previous 24 h), whereas previous exposures could not be traced. This fast kinetic may explain the results found in the Dutch bulb growers, for whom, although nominally exposed to EBDCs during the investigated period, an extremely low level of urinary ETU was observed (results not presented). Furthermore, through the determination of urinary ETU, we were able to measure detectable level of this substance in the majority of controls. This is in line with previous results on general population of Northern Italy, which showed that low ETU urine levels are detected in more than 50% of the general population not occupationally exposed to EBDCs or ETU.²³

Overall, no increased risk for exposed subjects for asthma and/or asthmatic symptoms, like wheeze and chest tightness was found. If any, we reported inverse associations for exposure to EBDCs and/or other pesticides and asthma and asthma-related symptoms. We did observe some slightly increased risk for asthma diagnosis and asthma attack adjusted for confounders, although none of these results were statistically significant, which might be due to the small number of cases.

Some other studies have reported positive associations for some dithiocarbamates but not all dithiocarbamates. Hoppin, *et al.*¹³ found a positive association for S-ethyl-dipropylthiocarbamate but not for other dithiocarbamate pesticides, like maneb and ziram.¹³ Cone, *et al.*²⁴ reported that residents exposed to

Table 2 Associations between exposure to pesticides and asthma outcome measurements for exposed vs non-exposed subjects. Data are shown as odds ratios with corresponding 95% confidence interval, crude or adjusted for potential confounders

	OR, 95% confidence intervals		
	Crude OR	Adjusted for field study	Adjusted for all confounders ^a
Asthma diagnosis			
Exposed to pesticides	0.37 (0.16–0.87)	0.37 (0.16–0.86)	0.41 (0.15–1.11)
Chest tightness			
Exposed to pesticides	0.57 (0.37–0.86)	0.53 (0.34–0.83)	0.60 (0.36–1.02)
Wheeze			
Exposed to pesticides	0.59 (0.38–0.94)	0.57 (0.36–0.91)	0.56 (0.32–0.98)
Asthma attack			
Exposed to pesticides	0.41 (0.12–1.35)	0.41 (0.13–1.37)	0.52 (0.12–2.25)
Asthma medication			
Exposed to pesticides	0.71 (0.26–1.94)	0.72 (0.26–1.97)	0.79 (0.25–2.53)

OR, odds ratio.

^aAssociation adjusted for age, residence, education, gender, smoking and field study.

metam sodium, a dithiocarbamate, as a result of chemical spill presented persistent irritant-induced asthma.

A possible explanation for the difference observed comparing the present research with previous studies may be linked to modalities of exposure, including multiple agents, differences in the active ingredients of pesticides and their amount, and application typology. For example, farmers are exposed to several agents that affect respiratory health other than chemicals (e.g. dust, molds, pollen, animal products, insects),^{11,12,25,26} whereas factory workers, like the group of investigated Bulgarian zineb producers, are mainly exposed to a specific pesticide.⁹ Moreover, agricultural workers are also exposed to some agents that may reduce the risk of asthma, for instance, contact with livestock and exposure to endotoxin.^{5,27,28}

We were able to control for some potential confounders (like education and smoking). However, our results are not likely to have been severely con-

founded due to the nature of exposure adjustment. Unlike, many other cross-sectional studies, we also measured exposure to dithiocarbamate by analyzing metabolites in urine (ETU). Despite the notable subject sample size, we had only a few asthmatic subjects, causing power difficulties. Thus, our estimates have a great random variation, as shown by the large confidence intervals. Unfortunately, we were also unable to study longitudinal effects because of lack of data on follow-up status with respect to asthma and/or asthmatic symptoms.

Furthermore, respiratory morbidity among agricultural workers could influence their ability to farm and may determine the chemicals they use and the crops and animals they raise. Subjects with severe respiratory diseases, like severe allergic asthma, are likely to avoid any kind of chemical exposure, and therefore, in the frame of the several different activities that an agricultural worker can do, ruling out pesticide application.^{13,25,26} We

Table 3 Univariate associations between urinary (ETU), as a marker of (EBDCs) exposure, and asthma outcome measurements for exposed versus controls

	Odds ratio per standard deviation (OR), 95% confidence intervals		
	Crude OR	Adjusted for field study	Adjusted for all confounders ^a
Asthma diagnosis			
Exposed to pesticides	0.92 (0.81–1.04)	1.14 (0.92–1.42)	1.19 (0.93–1.52)
Chest tightness			
Exposed to pesticides	0.99 (0.92–1.06)	0.90 (0.79–1.03)	0.90 (0.79–1.03)
Wheeze			
Exposed to pesticides	0.96 (0.89–1.04)	0.99 (0.88–1.13)	0.99 (0.87–1.13)
Asthma attack			
Exposed to pesticides	0.90 (0.72–1.11)	1.08 (0.72–1.62)	1.14 (0.72–1.80)
Asthma medication			
Exposed to pesticides	0.99 (0.86–1.14)	1.18 (0.95–1.48)	1.18 (0.93–1.50)

OR, odds ratio.

^aAssociation adjusted for age, residence, education, gender, smoking and field study.

could not determine whether asthmatics are less susceptible to the influence of chemical exposures or that sensitive asthmatics avoid chemical exposition. A further explanation for this finding could be the well-known lower incidence of allergic diseases in rural populations.^{29,30} In this light, the observed difference could be due to the distribution of exposed and controls between rural and urban area of residence, with a higher percentage of subjects living in urban environments among controls.

In conclusion, in general, we found no associations for exposure to EBDCs and other pesticides on asthma or asthmatic symptoms among exposed workers compared with non-exposed workers. However, we found slightly increased risks for asthma diagnosis and asthma medication for level of ETU, although none of these risks were statistically significant. Because of the small number of subjects with asthma and/or asthmatic symptoms in our study, we cannot consider the results of our study as conclusive because further research is needed to determine the effects of EBDCs and/or other pesticide usage on asthma and asthma-related symptoms.

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