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Occupational exposure to ethylenebisdithiocarbamates in agriculture and allergy: results from the EUROPIT field study

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This epidemiological study was carried out to evaluate the possible association between occupational exposure to ethylenebisdithiocarbamates (EDBC) and allergy. The study was conducted in four countries in the European Union: The Netherlands, Finland, Italy and Bulgaria. A total of 248 workers exposed to EDBC and 231 non-occupationally exposed subjects entered the study. Exposure to EDBC was measured as urinary ethylenethiourea (ETU) in urinary samples collected at baseline and after 30 days of exposure. Several effect parameters were evaluated including questionnaire data on allergy, Phadiatop, a general allergy test, and specific IgE parameters. These data were also collected at baseline and after 30 days of exposure. Cross-sectional as well as longitudinal comparisons were made, adjusted for potential confounding

factors. No association was found between exposure status, EDBC levels and allergic contact dermatitis, allergic rhinitis, food allergy or atopy as measured by the Phadiatop. The prevalence of skin irritation was elevated in the Dutch field study only and is more likely a result of plant contact rather than EDBC exposure. Occupational exposure to sunlight was noted to have a protective effect on atopy in terms of IgE positivity. We conclude that the EDBC exposure levels experienced in our field study are not associated with increased prevalence of allergic symptoms or allergy.

Key words: allergy; epidemiology; ethylenebisdithiocarbamate pesticides; fungicides; immunotoxicity; occupational exposure

Introduction

Dithiocarbamates are a group of pesticides widely used in crop protection because of their effectiveness against a wide spectrum of fungi. Other uses of these chemicals are found in sugar, paper and pulp manufacturing, as a vulcanization agent and antioxidant in rubber manufacturing. Dithiocarbamates comprise two subgroups: the ethylenebisdithiocarbamates, which includes zineb, maneb

and mancozeb, and the dimethyldithiocarbamates, which includes ferbam, ziram and thiram.¹

Allergy is a disproportional intensive immune response to an allergen. In respiratory allergy and food allergy, these responses are IgE-mediated, causing the release of histamine from mast cells or basophils. Contact dermatitis is mediated by cellular immune responses. Agricultural workers may come into contact with a range of well-established allergens such as allergens from plants, animal stock and moulds.²

Some investigators have hypothesized that exposure to pesticides may be associated with allergy.² However, the epidemiological evidence for such an association remains weak and rather applies to

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wheezing than allergy.^{3,4} Several cases of allergic responses to exposure to dithiocarbamates have been reported in the literature. Campbell and Forsyth⁵, for instance, reported two cases of allergic contact dermatitis following contact with maneb and/or zineb. Apart from these specific allergic reactions, it has also been postulated that some pesticides may have a more general effect on the immune system,^{6,7} but data are lacking and contradictory results reported.⁸ EDBC has been shown to be capable of inhibiting tumor necrosis factor (TNF) production in human CD4⁺ cells.⁹ A slight immunostimulator effect has been observed in workers occupationally exposed to EDBC.¹⁰ Later, the same research group reported increased CD19⁺ and decreased CD25⁺ cells in humans following exposure to mancozeb together with decreased TNF production.¹¹ These findings suggest that EDBC could have the potential deregulatory effect on the immune system in general, resulting in a slight immunostimulator activity that could bring about an increased expression of allergies in exposed individuals. However, overt changes in immune function following exposure to EDBC in terms of increased allergy rates have so far not been reported.

Our study was set up to study the possible effect of EDBC exposure on the immune system in occupationally exposed workers. In the context of this study, we also collected information on contact dermatitis, allergic rhinitis and food allergy.

Materials and methods

The EUROPIT study was designed to evaluate the potential immunologic effects of pesticide exposure, with a focus on EDBC. Five field studies were conducted in four countries. In the Netherlands, 42 flower bulb growers (mainly re-entry workers) and 40 non-occupationally exposed controls were examined. In Italy, the population consisted of 48 vineyard workers and 45 controls. The Finnish field study evaluated 51 potato farmers and 49 controls. One field study in Bulgaria studied 52 workers from a zineb producing facility and a second Bulgarian field study consisted of 55 greenhouse workers and 45 controls not occupationally exposed to EDBC. The controls were selected in such a way that optimal comparability was achieved in age distribution, gender, and educational level. Sampling was conducted in such a manner that an optimal comparability was achieved between exposed and controls. Overall, the total study population consisted of 248 EDBC exposed and 231 non-

occupationally exposed workers. The study was approved by the local Ethical Committees. All subjects agreed to participate in the study and signed an informed consent. Each field study was set up in such a way that baseline data on potential effect parameters were collected before the EDBC application season started (time T0) and that baseline data were collected in an as short as possible time frame. After a 1-month exposure period, a second wave of data collection took place in which the same parameters as at baseline were measured again (time T30). At the same time, exposure to EDBC was assessed based on the job description of each individual and by determination of urinary ethylenethiourea (ETU), one of the main metabolites of EDBC chemicals according to a previously described procedure.¹² ETU is thought to reflect short-term previous exposure because of its relatively short half-life. Even in homogenic exposure groups, individual ETU values have a substantial day to day variation because tasks and related exposure potential can vary significantly from day to day. Because of this substantial day to day variation, group mean ETU concentrations were regarded to be a more reliable exposure metric than the individual ETU values themselves.

ETU was determined at baseline and during the second wave of data collection. ETU concentrations were determined by means of GC/MS techniques. In addition, a self-administered questionnaire was used to collect information on job description, potential confounders including age, country, residence, education, gender, alcohol consumption, diet, medication use, smoking and sunlight exposure, potential effect parameters including skin irritation, contact dermatitis, allergic rhinitis and food allergy, and other diseases. The questionnaire was translated to Dutch, Finnish, Italian, and Bulgarian and later translated back to English to make sure that the translations were done correctly.

The functional assessment of the immune system was completed with the measure of the IgE concentration by ELISA. For specific IgE determination, 2 mL of blood sample was collected at the beginning of the study and after the 30-day exposure period (T30). Samples were stored at -80 °C before analyses. Samples were analyzed by Pharmacia Diagnostic. The Phadiatop test, a test to detect allergic reactions to common allergens, was used to inventory allergen specific responses. The allergens tested were cat, dog, house dust mite, mixture of grass pollen, mixture of tree pollen, mixture of herb pollen, and a mixture of fungi.

Statistical analysis

All data were analyzed using the SAS statistical package. The main statistical analyses consisted of comparisons between the exposed group at baseline or after exposure and the non-exposed group. Although the design of the study allowed for a longitudinal analysis (between baseline and second wave), a cross-sectional analysis was regarded to be more relevant for most of the effect parameters because for reported allergic complaints short-term changes were not regarded to be relevant. In addition, the results of the Phadiatop tests were prospectively analyzed. Analyses were also made in which ETU concentrations were used as exposure metric because there were substantial differences in ETU levels between the five field studies. The logarithm of the Phadiatop, a general allergy test, results and ETU level at the second wave of data collection (after exposure) and average logarithmic ETU level by job group were used as exposure metrics as the Phadiatop and ETU levels followed a log-normal distribution. For the ETU levels, Odds Ratios (ORs) were expressed per standard deviation of change in this level. 95% Confidence intervals (CIs) were calculated using the formula: $\text{Exp}(B/SD \pm 1.96 \times SE/SD)$. Adjustments for confounders were made by means of multivariate logistic regression modeling, for each potential effect parameter under investigation using the self-reported allergies as dependent variables. 95% CIs were calculated for the ORs. Statistical significance testing for the crude comparisons was done by using chi-square or the Student's *t*-test as indicated.

Results

Demographic variables and confounders

Given the agricultural character of the jobs of most exposed workers, the difference in rural/urban residence between the exposed group and the non-exposed group was not surprising (see Table 1). In addition, differences in education, ever having lived on a farm and sunlight exposure, are clearly related to the differences in jobs between the exposed and non-exposed groups. With respect to other variables, such as gender, smoking, alcohol consumption (not shown), age, and body mass index, the two groups were quite comparable.

EDBC exposure

The substantial difference in average urinary ETU levels between the exposed groups at time T30 compared either with baseline or the control group

Table 1 Distribution of the main characteristics in the exposed group and non-exposed group of the EUROPIT study

Variable	Non-exposed group (N = 231)	Exposed group (N = 248)
Average age	42.2	42.4
Gender (% men)	62.4	66.5
Residence (% urban)	63.8	37.4 ^a
Smoking (% ever)	57.6	56.6
Education		
Low (%)	9.7	33.3 ^a
Middle (%)	41.4	50.0
High (%)	48.9	16.7
Regular sunlight exposure (% yes)	66.5	78.8 ^b
Tan (%yes)	2.6	1.6
Ever lived on farm (%)	36.3	67.6 ^a
Average ETU in urine at <i>t</i> = T30 ^c	0.4	2.0 ^a
Average body mass index (kg/m ³ m ²)	25.3	25.8

ETU, ethylenethiourea.

^aStatistically significantly different at 0.001 level.

^bStatistically significantly different at 0.01 level.

^cGeometric mean values.

clearly reflect the difference in exposure levels. However, it must be noted that the ETU levels in the Dutch-exposed group were not different from the control group (0.81 and 0.81 µg/g creatinine). Highest ETU levels at T30 were observed in Bulgarian field study for the zineb factory workers, with an average of 22.12 µg/g creatinine in the factory workers and 17.01 µg/g creatinine in the control group (the two latter results are not shown).

Immune effects

The prevalences of immunological disorders are given in Table 2, and the results of the Phadiatop tests are given in Table 3. The prevalences of specific allergens included in the Phadiatop were similar between the exposed and non-exposed subjects. This was true for the comparison on the first wave of observations, and it was also true for the differences between the first and second wave. In addition, the

Table 2 Distribution of reported allergies in the exposed and non-exposed groups at baseline (T0)

Type of allergy reported	Non-exposed group (N = 231) % yes	Exposed group (N = 248) % yes
Skin irritation	13.2	17.0
Contact dermatitis ever	15.6	12.6
Contact dermatitis at	7.1	4.4
Allergic rhinitis, diagnosed	15.5	8.5 ^a
Sensitivity to grass pollen	13.3	8.1
Food allergy	8.2	5.1

^aStatistically significant at 0.05 level.

Table 3 Results of the Phadiatop test, for the exposed group and the non-exposed group at baseline (T0) and at the second interview (T30) in terms of IgE positivity to specific allergens

Allergen	Non-exposed (N = 231)		Exposed group (N = 248)	
	Baseline (T0)	Second wave (T30)	Baseline (T0)	Second wave (T30)
House dust mites (% pos)	29.3	30.3	25.3	25.3
Cat dander (% pos)	25.2	25.2	20.3	20.3
Dog dander (% pos)	23.2	23.2	21.2	21.2
Olive tree (% pos)	22.2	23.0	23.2	23.0
Grass pollen (% pos)	27.3	27.3	26.3	26.3
Fungi (% pos)	21.0	21.0	21.0	21.0
Trees (% pos)	27.2	27.2	23.3	23.3
Herbs (% pos)	22.2	22.2	23.2	23.2
Atopy (% pos)	26.2	29.2	22.3	22.3

prevalence of atopy was not statistically significantly different between the exposed and non-exposed. If anything, the prevalence of atopy was higher among the non-exposed than in the exposed. This was true for the first wave before the spraying season as well as for the second wave, 1 month into the application season.

Self-report information on the prevalence of skin irritation, contact dermatitis ever, current contact dermatitis, allergic rhinitis, sensitivity to grass pollen, and food allergy did not show any statistically significant association with exposure status, which did not change after adjustment for demographic variables or all confounders. In Table 4, the crude and adjusted ORs are presented for the association between EDBC exposure and allergic symptoms. Apart from exposure status based on the sampling scheme of the study, ETU in urine samples were determined for each study subject. However, we were aware that ETU concentrations in urine only provide information on recent exposure, which may not be representative for prolonged exposure conditions. We, therefore, considered the average ETU levels for each job as being a robust estimate of EDBC exposure during the whole study period, whereas we used the data collected from the questionnaire to assess historical exposure to pesticides for the subjects in the study. The group urinary ETU averages per job were entered into the logistic

regression model as exposure metric. This model was used to investigate dose-response relationships. ORs per standard deviation of difference were calculated, with and without adjustment for confounders (see Table 5). Again, no significant associations emerged between ETU level grouped by job and allergies.

To assess the short-term effect of exposure to EDBC pesticides, we evaluated the changes between (pre-exposure) baseline levels and after the 1-month exposure period. These comparisons were adjusted for residence, gender, smoking, education, and sunlight exposure by means of logistic regression. No significant association was found between exposure status and level of EDBC exposure as urinary ETU. The only significant association that emerged was a decrease in IgE status and sunlight exposure (see Table 6). We observed an effect on IgE levels from sunlight exposure, with lower IgE levels in individuals reporting sunlight exposure for more than 5 h/week. This finding is in line with experimental work done by van Loveren and Boonstra (2000).

Discussion

The results of this study indicate that exposure to EDBC does not bring about an increased prevalence of allergic contact dermatitis, allergic rhinitis, food

Table 4 Odds ratios (OR) and 95% confidence intervals (CI) for the associations between EDBC pesticides and self reported allergy, contact dermatitis, allergic rhinitis and food allergy baseline or after exposure

Type of allergy	Crude OR (95% CI)	OR (95% CI) adjusted for demographic variables ^a	OR (95% CI) adjusted for all potential confounders ^b
Skin irritation	1.34 (0.80–2.24)	1.41 (0.78–2.53)	1.36 (0.73–2.52)
Contact dermatitis	0.83 (0.50–1.38)	0.92 (0.51–1.66)	0.99 (0.53–1.86)
Allergic rhinitis	0.50 (0.28–0.89)	0.58 (0.30–1.10)	0.76 (0.37–1.56)
Food allergy	0.71 (0.35–1.43)	0.70 (0.30–1.62)	0.79 (0.32–1.91)

^aAdjusted for age, gender, residence, and education.^bAdjusted for age, gender, residence, education, smoking, field study, and sunlight exposure.

Table 5 Odds ratios (OR) and 95% confidence intervals (CI) per standard deviation of log ethylenethiourea urine level and self-reported skin irritation, contact dermatitis, allergic rhinitis, and food allergy

Type of allergy	Crude OR (95% CI)	OR (95% CI) adjusted for demographic variables ^a	OR (95% CI) adjusted for all potential confounders ^b
Skin irritation	0.80 (0.58–1.09)	0.84 (0.60–1.17)	0.95 (0.48–1.88)
Contact dermatitis	0.88 (0.77–1.03)	0.94 (0.81–1.10)	0.99 (0.82–1.21)
Allergic rhinitis	0.92 (0.79–1.07)	0.92 (0.78–1.08)	1.02 (0.81–1.29)
Food allergy	1.04 (0.89–1.22)	1.00 (0.83–1.20)	1.13 (0.89–1.43)

^aAdjusted for age, gender, residence, and education.^bAdjusted for age, gender, residence, education, smoking, field study, and sunlight exposure.

allergy, or atopy in exposed workers. Adjustment for confounders did not change these findings. Conversely, these conditions were slightly inversely associated with exposure to EDBC, with the exception of skin irritation which had a higher prevalence in the exposed group as compared with controls. However, this excess was mainly restricted to the field study in The Netherlands with the lowest exposure to EDBC, which involved flower bulb re-entry workers with prolonged hand contact to plants. In the Dutch field study, 28.6% of the exposed workers reported skin irritation as compared with 7.7% in the non-exposed group at baseline, a statistically significant difference. Flower bulb re-entry workers come in contact with plant leaves during most of the growing season and it is plausible that repeated plant contact may be the cause of the reported skin irritation. Skin irritation was measured by means of a self-administered questionnaire, which is thought to be less reliable than clinical examination. However, a clinical examination would only detect skin irritation at the time of examination. Questionnaire can cover longer periods of time. It is less likely that EDBC are involved because the Dutch field study was the study with the lowest urinary ETU concentrations in post-exposure samples of all five conducted field studies. In fact the average ETU levels were similar for exposed as for non-exposed individuals in that field study. We found a statistically significant lower prevalence of rhinitis in the exposed group than in the non-exposed group. This finding

persisted after adjustment for age, gender, education, and residence. It is not clear from our study which underlying factors contribute to the lower prevalence. It is possible that the exposed group had experienced lower exposure to general allergens or lower levels of air pollutions. Our study was not designed to investigate all potential sources of allergy. It rather focused on the association between EDBC exposure and allergy.

This analysis covers a substantial range of occupational exposure to EDBC as reflected in ETU levels. For instance, the average ETU level in the Dutch re-entry workers was very low, and similar to the control group. It is likely that exposure in the Dutch re-entry workers was very low, although a substantial segment of the working hours are spent in the flower bulb field at the time of the urine sample collection. In addition, the re-entry workers in The Netherlands are advised to avoid skin contact with plants and bulbs as much as possible to avoid pesticide uptake. However, in the workers of a zineb production facility in Bulgaria, a 20-fold average ETU level was found as compared with the Dutch re-entry workers. We, therefore, consider the range of exposure in the total study to be substantial. The analyses based on ETU level still do not indicate an effect of EDBC exposure in terms of increased prevalence of atopy, skin irritation, allergic contact dermatitis, allergic rhinitis, contact dermatitis, or food allergy.

Although the EUROPIT study was specifically designed to observe short-term effects of EDBC exposure on the immune system, it also provides evidence for potential long-term effect because a large proportion of the workers enrolled in the study had been employed at their current job for a number of years before the study.

In summary, we conclude that under the conditions under investigation exposure to EDBC does not affect the immune balance of these workers, and that the slight immune changes observed in previous studies after short-term exposure to EDBC

Table 6 Results of the linear regression analysis showing changes in log (IgE) status from baseline to 30 days adjusted for field study and exposure status

Parameter	Change in log(IgE) level
Exposure status (exposed versus non-exposed)	-0.219
Residence (specify categories)	0.064
Gender (specify categories)	0.385
Smoking status (smokers versus non-smokers)	0.357
Education (specify categories)	0.147
Sunlight exposure (>5 h/week)	-1.051 ^a

^aStatistically significant at 0.05 level *P* value <0.05.

can be considered adaptive and not to bring about any significant risk immunotoxic effects.

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References

- 1 Liesivuori, J, Savolainen, K. Dithiocarbamates. *Toxicology* 1994; **91**: 37–42.
- 2 Heederik, D, Sigsgaard, T. Respiratory allergy in agricultural workers: recent developments. *Curr Opin Allergy Clin Immunol* 2005; **5**: 129–134.
- 3 Hoppin, JA, Umbach, DM, London, SJ, Alavanja, MCR, Sandler, DP. Chemical predictors of wheeze among farmer pesticide applicators in the Agricultural Health Study. *Am J Respir Crit Care Med* 2002; **165**: 683–689.
- 4 Hoppin, JA, Umbach, DM, London, SJ, Alavanja, MCR, Sandler, DP. Pesticides associated with wheeze among commercial pesticide applicators in the Agricultural Health Study. *Am J Epidemiol* 2006; **163**: 1129–1137.
- 5 Campbell, FA, Forsyth, A. Dithiocarbamate-induced allergic contact dermatitis. *Contact Dermatitis* 2003; **49**: 305–306.
- 6 Colosio, C, Corsini, E, Barcellini, W, Maroni, M. Immune parameters in biological monitoring of pesticide exposure: current knowledge and perspectives. *Toxicol Lett* 1999; **108**: 285–295.
- 7 Colosio, C, Birindelli, S, Corsini, E, Galli, CL, Maroni, M. Low level exposure to chemicals and immune system. *Toxicol Appl Pharmacol* 2005; **207**: 320–328.
- 8 Botham, PA. Are pesticides immunotoxic? *Adverse Drug React Acute Poisoning Rev* 1990; **9**: 91–101.
- 9 Pyatt, DW, Gruntmeir, J, Stillman, WS, Irons, RD. Dimethyldithiocarbamate inhibits in vitro activation of primary human CD4+ T lymphocytes. *Toxicology* 1998; **128**: 83–90.
- 10 Colosio, C, Barcellini, W, Maroni, M, Alcini, D, Bersani, M, Cavallo, D, et al. Immunomodulatory effects of occupational exposure to mancozeb. *Arch Environ Health* 1996; **51**: 445–451.
- 11 Corsini, E, Birindelli, S, Fustinoni, S, De Paschale, G, Mammone, T, Visentin, S, et al. Immunomodulatory effects of the fungicide Mancozeb in agricultural workers. *Toxicol Appl Pharmacol* 2005; **208**: 178–185.
- 12 Fustinoni, S, Campo, L, Colosio, C, Birindelli, S, Foà, V. Application of gas chromatography-mass spectrometry for the determination of urinary ethylenethiourea in humans. *J Chromatogr B Analyt Technol Biomed Life Sci* 2005; **814**: 251–258.
- 13 Van Loveven, H, Boonstra, A. UV exposure alters respiratory allergic response in mice. *Photochem Photobiol* 2000; **72**: 253–259.