Thyroid Hormones and Cytogenetic Outcomes in Backpack Sprayers Using Ethylenebis(dithiocarbamate) (EBDC) Fungicides in Mexico

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Ethylenebis(dithiocarbamate) (EBDC) fungicides are used heavily in the United States. EBDCs (e.g., mancozeb, maneb) are metabolized to ethylene thiourea (ETU). The EPA classifies ETU as a carcinogen, based on thyroid and other cancers in rodents, and has restricted the use of EBDCs, while requiring workers to use protective equipment. There are no data on the potential carcinogenicity of EBDCs in humans, and there is only one study on human genotoxicity. ETU is known to cause decreases of thyroxine (T_4) and increases in thyroid-stimulating hormone (TSH) in rodents. We have studied cytogenetic outcomes and serum thyroid hormone levels among 49 heavily exposed workers without protective equipment spraying EBDC on tomatoes in Mexico. We also studied 14 lightly exposed landowners and 31 nonexposed controls. Urinary ETU was used to compare exposure between groups. We found an increase in TSH ($p = 0.05$) among applicators compared to controls, but no decrease in thyroid hormone (T_4). We found increases in sister chromatid exchange ($p = 0.03$) and in chromosome translocations (chromosome aberrations that persist through cell division) for applicators compared to controls ($p = 0.05$). However, the subset of reciprocal translocations showed a lesser increase ($p = 0.24$). Our data suggest that EBDCs affect the thyroid gland and the lymphocyte genome among heavily exposed workers. However, our data are limited to subclinical outcomes, are of borderline statistical significance, and should be interpreted with caution. *Key words*: cytogenetics, ethylenebis(dithiocarbamates), EBDCs, ethylene thiourea, ETU, pesticides, thyroid.
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Ethylenebis(dithiocarbamate) (EBDC) fungicides (maneb, mancozeb, and metiram) are among the most common in the United States, with approximately 8–12 million pounds of active ingredients used annually prior to 1990 (*1*). Different EBDCs share a common molecular structure, but differ by metal cation (Fig. 1). EBDCs are absorbed primarily dermally and are metabolized to ethylene thiourea (ETU). Dermal absorption of EBDCs range from 1 to 10%, and approximately 7.5% of the absorbed dose is converted to ETU (*1*). EBDCs and ETU are mildly genotoxic in bacterial and animal systems (*1*). The EPA classifies ETU as a carcinogen, based on animal data showing that it causes thyroid and other cancers in rodents. Because of concern about carcinogenicity, in 1992 the EPA canceled the use of EBDCs on 11 crops, but EBDCs are still used in the United States on a wide variety of fruit, nut, and vegetable crops such as apples, almonds, and tomatoes (*1*). There are no data on the potential carcinogenicity of EBDCs in humans. In the one study on human genotoxicity, 44 workers producing a fungicide containing mancozeb showed significantly increased chromosomal aberrations (CAs; primarily breaks) in exposed nonsmokers compared to controls and nonsignificant elevations among exposed smokers compared to controls (*2*). CAs are visible at metaphase as chromosomal breaks or rearrangements and are often lethal to the cell. These same data showed nonsignificant elevations of sister chromatid exchanges (SCEs) of exposed versus nonexposed workers after stratification by smoking.

ETU has also been shown to cause a significant decrease in thyroxine (T_4) in production workers (mixers) who were highly exposed versus controls, although the number of mixers was small and calculated variances of T_4 levels do not appear to have taken into account that there were repeated measures on the same person (*3*). ETU has been shown to have the same effects in rodents, causing a decrease in T_4 and an increase in thyroid-stimulating hormone (TSH). It has been hypothesized that this disruption of thyroid homeostasis, which correlated with morphological changes in the thyroid gland, may be related to the thyroid tumors observed in rodents (*4*).

We sought to investigate further the possible cytogenetic and thyrotoxic effects of EBDC fungicides. Our study population was composed of Mexican backpack sprayers applying EBDCs to tomatoes, a group of lesser exposed landowners, and a nonexposed comparison population. Urinary ETU was measured to evaluate current exposure. Cytogenetic outcomes included sister chromatid exchange and chromosome aberrations. The latter outcome was measured by chromosome painting, which enables detection of stable aberrations such as translocations, as well as unstable events such as dicentrics and acentric fragments.

Materials and Methods

Selection of subjects. Data were collected on a population of 49 backpack applicators, 14 small landowners, and 31 nonexposed controls who were employed by city government in construction jobs. Blood samples for hormone analysis and questionnaires were also collected from an additional group of 14 controls to replace two shipments of blood, which were delayed in shipment and were damaged because they arrived without ice.

All subjects were males and lived near Cuernavaca in central Mexico. Subjects were selected via contact with small landowners who grow tomatoes in the area. All workers employed by a recruited landowner were also recruited, and recruitment continued until the target sample size of 50 exposed subjects was assured.

Demographic data were collected by questionnaire, including data on smoking and alcohol use. All subjects were compensated $10 for their time and inconvenience, and all subjects signed consent forms explaining the study. All blood was drawn in the morning.

The backpack applicators applied pesticides seasonally (June to November), primarily to tomatoes. Many applicators were migrants who returned to their home province for part of the year. Owners were full-time tomato farmers and were generally present during application; owners were likely to have some exposure, although less than the applicators.

Exposure. EBDCs were applied to tomatoes in a liquid mixture of water, plant nutrients, and other pesticides as needed. These other pesticides typically included an organophosphate, e.g., chlorpyrifos or methamidophos, as well as foliar nutrients and growth regulators. On occasion another

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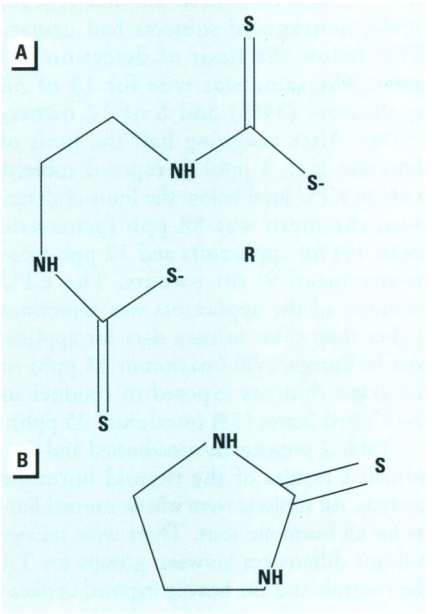

Figure 1. A) Structure of maneb and mancozeb (chelate and metal). B) Structure of ethylene thiourea (ETU). Maneb, R = Mn^{2+}; mancozeb, R = Mn^{2+} + Zn^{2+}.

fungicide, captan, was used along with the EBDCS, but this was not done commonly. A typical mixture included 0.75 kg mancozeb (powder), 0.33 liter liquid methamidophos (48% by weight), 1 kg plant regulator (powder), 0.25 liter foliar nutrients (liquid), and 200 liters water in a large barrel. Mixing was done without protective equipment; the pesticides were poured into the mix by hand and then stirred with a stick. A bucket was then dipped into the mixture, filled, and poured into backpack sprayers containing approximately 20 liters. Spills onto the skin were common in this process. Crews typically had three to six members. Depending on the size of the field, workers might apply 5–10 barrels a day over a period of perhaps 4–6 hr. Use of fungicides varied with the amount of rain; wetter conditions required more fungicides. Applicators wore only light clothing and sandals, and a fine spray of pesticides generally drifted onto the clothes and exposed body surfaces of the applicators. Each crew, typically employed by the owner over the season, applied pesticides once or twice a week.

Collection of biological samples. Different numbers of subjects were available for analysis of different endpoints. Blood was drawn in the morning for both exposed and nonexposed subjects. All blood samples were shipped on ice within 24 hr to the laboratory. For the exposed subjects, an attempt was made to collect blood in the morning on a day exposure took place and to collect the first-void urine the morning of the next day from the same worker. The urinary elimination half-life of ETU in humans has been estimated to be approximately 32–100 hr, with highest levels excreted in the urine in the first 24 hr after exposure (*4,5*).

Most subjects contributed both urine and blood; however, some subjects contributed only one or the other (no subject contributed more than one sample for either blood or urine). The urine was used to confirm exposure and to serve as a basis of comparison with other studies (ETU is generally not present in the urine of individuals without EBDC exposure). Urinary ETS was not used for exposure–response analyses, so having blood and urine samples on the same people was not essential.

Serum thyroid hormone data were originally available on all 31 nonexposed subjects and 51 of 63 exposed subjects. Because two shipments of blood (21 nonexposed, 8 exposed subjects) were delayed and arrived after ice melted in hot summer temperatures (altering hormone levels), these samples could not be used for the hormone analysis, although most were able to be used for cytogenetic analysis. Fourteen additional controls were then recruited, and blood samples were obtained from them for hormone analysis only. Cytogenetic data were available for 30 of 31 nonexposed subjects and 44 of 63 exposed subjects. Urine data were available for all 31 nonexposed subjects and for 50 of 63 exposed subjects. Data were missing for a variety of reasons. Some subjects refused to supply the sample (typically the blood), others failed to collect and/or deliver the sample (typically the urine), and some samples were inadvertently lost in transport (blood).

Urine samples were stored on dry ice and shipped to the lab. Double blind samples spiked with known amounts of ETU were also sent to the lab for quality control and confirmation of the limit of detection. Analysis of urine for ETU was done via high performance liquid chromatography using an electrochemical detector (*6*), with a limit of detection of 10 ppb.

Blood samples for thyroid hormone analysis were collected in 5-ml serum separation tubes and spun down to separate the erythrocytes prior to shipping. Both T_4 and TSH were analyzed via enzyme immunoassays (MEI or microparticle enzyme assay for TSH via IMX, CEDIA enzyme immunoassay for T_4). Blood samples for cytogenetic analysis were collected in 7-ml heparinized tubes.

Cytogenetic procedures. Cell culture and cytogenetic analyses were conducted in Livermore, California, without knowledge of the exposure category of samples. Whole blood (0.8 ml) was added to 10 ml of RPMI 1640 medium (Gibco/BRL, Gaithersburg, MD) supplemented with 15% fetal bovine serum, 100 IU/ml penicillin, 100 µg/ml streptomycin, 1% sodium heparin, 2% phytohemagglutinin, and 2 mM L-glutamine. Four cultures were prepared for each subject, two of which were supplemented with 100 mM bromodeoxyuridine (BrdU) to obtain SCEs. Cultures were incubated at 37°C in a 5% CO_2 environment. The culture duration was 52 and 72 hr for aberrations and SCEs, respectively; in each case, 0.1 µg/ml Colcemid was added for the last 4 hr of culture. Cells were harvested by swelling in 0.075 M KCl and then fixed a minimum of three times in 3:1 (v/v) methanol:glacial acetic acid. Well-spread metaphase cells, low in cytoplasm, were prepared as described elsewhere (*7*). All slides were stored at -20°C in sealed plastic bags in the presence of a desiccant and N_2 until needed for hybridization.

For SCEs, slides were stained with the fluorescence-plus-Giemsa method (*8,9*). Fifty second-division cells were scored, 25 from each of two replicate cultures. Two readers each scored one culture per donor. Most cells had 46 chromosomes, although some had as few as 45 or as many as 48 chromosomes. The SCEs were normalized to 46 chromosomes per cell prior to analysis.

For chromosome painting, slides were hybridized with SpectrumOrange chromosome-specific DNA probe (Vysis, Downers Grove, IL) for chromosomes 1, 2, and 4 simultaneously according to the manufacturer's instructions, with minor modifications as described elsewhere (*7,10*). The slides were mounted in a DAPI antifade solution (Sigma, St. Louis, MO) (*11*) and coded. All cells were visualized with a dual band pass filter (Vysis), which allows simultaneous viewing of SpectrumOrange labeled and DAPI counter-stained chromosomes. Metaphase cells were considered scoreable if they met the following criteria: 1) the cells appeared intact with well-spread chromosomes, 2) the centromeres of all chromosomes were readily visible, 3) the centromeres from all six painted chromosomes were present, and 4) the SpectrumOrange label was sufficiently bright to detect exchanges between painted and unpainted chromosomes. Every abnormal metaphase cell was photographed with Kodak Ektachrome 400 film (Eastman Kodak, Rochester, NY). These photographs provide a permanent record of each aberrant cell and were used to resolve ambiguous aberrations. All aberrant cells were categorized according to the Protocol for Aberration Identification and Nomenclature Terminology (PAINT) (*12*). Following the initial evaluation of aberrations, translocations were reclassified as apparently reciprocal if two color junctions, in one each of two

chromosomes, were visible (*13*). If only one color junction was evident, the translocation was considered to be nonreciprocal. Analyses were conducted for all translocations and for the subset of reciprocal translocations.

The analysis of aberrations by chromosome painting involves several assumptions (*14*). With painting, only a fraction of all events are seen, specifically, only those with exchanges occurring between painted and unpainted chromosomes. The fraction of all translocations detected by hybridization (F_h) is determined from the amount of the genome painted and is related to the fraction observable by G-banding (F_b) by the equation

$$F_h = 2f_s(1-f_s)F_b$$

where f_s is the proportion of the genome painted (*13*) and F_b is assumed to be 1. The fraction of all dicentrics detected is determined by the same formula. In the work presented here, chromosomes 1, 2, and 4 were painted simultaneously. These chromosomes represent 22.05% of the genome (*15*) and detect 34.4% of all exchanges. In this study, approximately 1500 metaphase cells were scored per subject. This is equivalent to 1500 × 0.344 = ~500 metaphase cells if the full genome were scored (defined as cell equivalents).

Statistical analysis. Statistical analysis for the hormone and SCE data was done by ordinary linear regression analyses, and least square means for exposure groups were calculated, adjusted for covariates. Residuals for these models were approximately normal. Analysis of the chromosome aberration data was done via Poisson regression, as positive outcomes were rare events in these data. The number of cell equivalents was taken into account by including it as an offset (constant term) in the Poisson regression; results for exposure effect are invariant to proportional changes in this offset and are therefore independent of our assumptions regarding cell equivalents. Age (categorized by 5-year intervals: <20, 20–24, 25–29, 30–34, 35–39, >40) was included as a covariate in all regressions for hormone outcomes, SCEs, chromosome translocations, and reciprocal translocations. Other aberrations were rare (making a model with fewer parameters preferable) and have been shown to increase approximately linearly with age (*7*). Therefore, models for dicentrics, insertions, fragments, and rings included exposure and a single (continuous) parameter for age.

There were only seven subjects over 40 years of age in the study, and six of these were exposed. Given this imbalance in the highest age category and because of the known importance of age for several outcomes, particularly chromosome translocations (*16*), we also conducted additional analyses for all outcomes after restricting the subjects to those aged 40 or less.

Smoking was thought *a priori* to be a potential confounder for sister chromatid exchange and, to a lesser extent, for chromosomal translocations. A dichotomous variable for current smoking was added to models for all outcomes and tested. It was not a significant predictor in any model, possibly because smokers in this population were very light smokers (4–5 cigarettes/day); therefore, the smoking variable was dropped for all models but the SCE analysis. It was retained in the final model for SCEs because it had some predictive value ($p = 0.16$) and some slight confounding effect on the exposure effect. Alcohol consumption was also tested for both SCEs and CAs, but was not an important predictor for either outcome; it was not included in final models.

Analyses were conducted using SAS (PROC GLM or PROC REG for linear regression, PROC GENMOD for Poisson regression; SAS Institute, Cary, NC) (*17*).

Results

Table 1 gives the results for age, smoking, and urinary ETU by three exposure categories (nonexposed, owners, applicators). The exposed applicators and owners were somewhat older, on the average, than the nonexposed. The majority of all groups smoked, but smokers smoked few cigarettes (subjects who smoked one cigarette or less per week were considered nonsmokers). All of the nonexposed subjects had urinary ETU below the limit of detection (10 ppb). The same was true for 13 of 38 applicators (34%) and 6 of 12 owners (50%). After assigning half the limit of detection (i.e., 5 ppb) to exposed subjects with an ETU level below the limit of detection, the mean was 58 ppb (geometric mean 19) for applicators and 12 ppb (geometric mean 9) for owners. The ETU exposure of the applicators was somewhat higher than prior urinary data for applicators in Europe (*18*) (maximum 13 ppb) or for grape thinners exposed to residues in the United States (*19*) (maximum 25 ppb).

Table 2 presents the unadjusted and age-adjusted results of the thyroid hormone analysis. All subjects were within normal limits for all hormone tests. There were no significant differences between groups for T_4: the controls and the heavily exposed applicators had virtually identical results. The unadjusted data also showed no significant differences for TSH (applicators compared to nonexposed, $p = 0.20$). Age was negatively correlated with TSH and acted as a negative confounder in these data. The data adjusted for age indicated an exposure effect. The applicators had a significantly higher amount of TSH (0.52 mIU/l, $p = 0.05$) than the nonexposed, while the landowners had a minimal and nonsignificant elevation of TSH (0.13 mIU/l, $p = 0.74$). Increased TSH may indicate hypothyroidism even in the absence

Table 1. Demographic variables and urinary ethylene thiourea (ETU) for study subjects

	Applicators ($n = 49$)	Owners ($n = 14$)	Nonexposed ($n = 31$)
Mean age ± SE	26.2 ± 1.6	31.6 ± 2.5	22.0 ± 1.2
Smokers (%)	70	50	67
Cigarettes/day smokers ± SE	3.5 ± 0.8	4.8 ± 1.3	5.1 ± 2.5
Mean urinary ETU ± SE (ppb)[a]	58 ± 26	12 ± 3	NA
Geometric mean urinary ETU (geometric SD)	19 (4)	10 (2)	NA

Abbreviations: SE, standard error; SD, standard deviation; NA, not available.
[a]Urine samples were available for 38 applicators, 12 owners, and 31 nonexposed subjects. All nonexposed subjects had levels below limit of detection (10 ppb) as did 34% of applicators and 50% of landowners. Samples for exposed subjects below the limit of detection were assigned half the limit of detection.

Table 2. Thyroid hormone results[a]

	Applicators ($n = 32$)	Owners ($n = 11$)	Nonexposed ($n = 24$)
Unadjusted for age			
Mean T_4 ± (μg/dl)[b]	7.65 ± 0.18	8.11 ± 0.43	7.68 ± 0.41
Mean TSH ± SE (mIU/l)	2.09 ± 0.18	1.45 ± 0.23	1.79 ± 0.13
Adjusted for age (5 categories)[c]			
Mean T_4 ± SE (μg/dl)	7.68 ± 0.22	8.24 ± 0.43	7.59 ± 0.29
Mean TSH ± SE (mIU/l)	2.13 ± 0.15*	1.73 ± 0.30	1.61 ± 0.19

Abbreviations: SE, standard error; mIU, milli-International unit; T_4, thyroxine; TSH, thyroid-stimulating hormone.
[a]Laboratory reference ranges for T_4 and TSH were 4.5–11.5 and 0.32–5.00, respectively. Four subjects were excluded from hormone analyses because the lab reported hemolysis, which might have affected results.
[b]SI unit conversion: μg/dl = 12.9 nmol/l.
[c]Least square means adjusted for age (categorical); adjustment for age using a continuous variable yielded similar results.
*Significantly increased compared to the nonexposed; $p = 0.05$.

of a decrease in T_4 levels (*20*), although in this population, TSH levels are all within normal range. The linear regression model for TSH and T_4, including exposure and age variables, explained 23% of the variance (r^2) for TSH, but only 3% for T_4. Adjustment for age using a continuous instead of a categorical variable showed similar results for TSH (difference between applicators and nonexposed, $p = 0.03$). Restriction of the data to those under 30 years of age (87% of applicators and nonexposed controls) showed a similar pattern of higher TSH among the applicators in the data with no age adjustment (2.25 µg/dl for applicators; 1.81 µg/dl for controls, $p = 0.07$).

Table 3 presents the cytogenetic outcomes by exposure group. Both unadjusted and adjusted (for age, smoking, via linear regression) data indicated that the applicators had significantly higher SCEs than nonexposed controls (0.62 increase in SCE per cell, $p = 0.02$, adjusted data). Owners were not significantly different from controls. The proportion of variance explained by the regression model was 22%.

Table 3 also presents the unadjusted and age-adjusted results for chromosomal aberrations. The unadjusted data show significant differences between applicators and owners compared to nonexposed controls for translocations and for the subset of reciprocal translocations. However, translocations increase markedly with age, and age acts as a confounder in these data. The Poisson regression analyses controlling for age indicated that applicators had significantly more total translocations than did the nonexposed subjects (1.50 increase, $p = 0.05$), while owners had a nonsignificant increase (0.85 increase, $p = 0.31$). Additional analyses using terms for age and age squared to control for age, rather than age categories, yielded essentially the same results. Analyses restricted to those age 40 and below resulted in a slightly lower predicted increase for applicators (1.25 translocations) compared to the nonexposed ($p = 0.14$). Analyses restricted to reciprocal translocations also yielded a lower and nonsignificant exposure effect for applicators versus the nonexposed ($p = 0.24$).

Poisson regression analyses of other chromosome outcomes (dicentrics, insertions, fragments, and rings) did not show any significant differences between the exposed group and the nonexposed referents.

Total translocations and reciprocal translocations were both significantly correlated with SCEs, with Spearman correlations of 0.28 ($p = 0.02$) and 0.27 ($p = 0.02$), respectively.

Discussion

We have studied two groups of subjects exposed to EBDCs during application to tomatoes in Mexico. Urinary ETU data confirmed that the applicators were more highly exposed than the landowners, while all nonexposed subjects had urinary ETU levels below the limit of detection. The fact that one-third of the applicators and one-half of the landowners had urinary ETU levels below the limit of detection can be explained by 1) a lack of sensitivity of the assay to detect low ETU levels, even when there was exposure; 2) differences in metabolism of EDBCs to ETU between subjects; and 3) different work practices and cleanup practices between subjects. Landowners generally had less contact with the pesticides, and it is perhaps not surprising that half had urinary ETU below the limit of detection. Among the applicators, division of the population by number of baths per week showed that those who bathed daily had a higher proportion of urinary ETU samples below the limit of detection, and had significantly lower mean urinary ETU, than those who bathed less often (data not shown). Another possible reason for urinary data below the limit of detection is misclassification; the urine collection was not observed directly and some samples could have been for the wrong person.

Although all the thyroid hormone data were within normal range, after age-adjustment the TSH level was significantly increased in the applicators compared to the nonexposed subjects (and nonsignificantly increased among the landowners, suggesting a dose response). In our data, age was negatively correlated with TSH and acted as a negative confounder. Several reports in the literature indicate that TSH levels decrease with age in healthy populations (*21,22*), but other studies have not shown such an effect (*23*). The exposure effect for applicators versus nonexposed controls for TSH was also apparent without age adjustment ($p = 0.07$) when the data were restricted to younger subjects (<30 years of age, 87% of applicators and controls).

Because T_4 levels were not decreased among the exposed compared to the nonexposed, TSH may be a more sensitive indicator of an effect on the thyroid than T_4 levels (*20*). Most of these workers had been exposed to EBDCs and ETU for several weeks or months at the time of testing; homeostasis with normal T_4 but elevated TSH might be expected after several weeks of exposure (*24*). The effect on TSH is consistent with animal data (*1*). In the one study of ETU-production workers with presumed high exposure, the five most highly exposed mixers had significantly decreased T_4 and one had abnormally high TSH with clinical signs of premyxedema (*3*). We did not observe any clinical signs of hypothyroidism in our study population, but we performed no clinical exams. If hypothyroidism does occur in these workers, it is possible that

Table 3. Cytogenetic outcome results[a]

	Applicators ($n = 31$)	Owners ($n = 13$)	Nonexposed ($n = 30$)
SCEs per cell			
Unadjusted	8.50 ± 0.22*	7.91 ± 0.21	7.80 ± 0.18
Adjusted for age, smoking[b]	8.52 ± 0.19*	7.61 ± 0.31	7.90 ± 0.20
Chromosome aberrations			
Unadjusted			
All translocations	4.61 ± 0.94	6.00 ± 1.00	2.76 ± 0.54
Reciprocal translocations	2.70 ± 0.53	3.38 ± 0.65	1.73 ± 0.32
Dicentrics	0.42 ± 0.18	0.30 ± 0.13	0.23 ± 0.10
Insertions	0.06 ± 0.04	0.15 ± 0.10	0.03 ± 0.03
Rings	0.10 ± 0.05	NA	0.13 ± 0.08
Fragments	0.87 ± 0.2	1.07 ± 0.31	0.76 ± 0.20
Adjusted for age[c]			
All translocations	6.07 ± 0.97**	5.44 ± 1.00	4.59 ± 0.82
Reciprocal translocations	3.39 ± 0.74	2.92 ± 0.72	2.71 ± 0.65
Dicentrics	0.40 ± 0.30	0.26 ± 0.13	0.23 ± 0.09
Insertions	0.06 ± 0.04	0.11 ± 0.08	0.03 ± 0.04
Rings	0.08 ± 0.05	NA	0.13 ± 0.07
Fragments	0.81 ± 0.15	0.81 ± 0.22	0.76 ± 0.16

Abbreviations: SCEs, sister chromatid exchanges; SE, standard error; NA, not available.
[a]Values given are mean ± SE.
[b]Adjusted SCE results are least-square means.
[c]Chromosome aberrations per 500 cell equivalents. The adjusted data chromosome translocations and the subset of reciprocal translocation are predicted from a Poisson regression model with exposure and age (five categories), while other aberration data uses a model with exposure and age as a continuous variable. Predicted results are for a subject 25–30 years of age. Variance of predictions derived from the standard error of Xβ using the delta method.
*Significantly increased compared to the nonexposed; $p = 0.03$ for adjusted data and $p = 0.04$ for nonadjusted data.
**Significantly increased compared to the nonexposed, $p = 0.05$.

affected subjects could not continue in this type of relatively strenuous work.

Our data on genotoxic outcomes indicate that the applicators had significantly increased SCEs, with or without adjustment for age and smoking. Chromosome translocations were significantly elevated for both applicators and owners ($p<0.001$) before age adjustment, compared to nonexposed subjects. However, age acted as a positive confounder in these data; after age adjustment, the exposure effect was diminished, although it was still present for applicators versus nonexposed controls ($p = 0.05$). When analyses were restricted to the subset of reciprocal translocations, the increase for applicators failed to reach conventional statistical significance after age adjustment ($p = 0.24$). In the age-adjusted data, again the landowners showed less pronounced elevations than applicators, suggesting a possible dose response. Reciprocal translocations are thought to be more stable than nonreciprocal translocations (*13*), and the somewhat more pronounced effect seen for all translocations (including nonreciprocals) may reflect the effects of more recent exposure. Chromosome outcomes other than translocations did not significantly differ between the exposed subjects and controls, but most of these outcomes were quite rare and results lack statistical precision.

The increased SCEs indicate that exposure is affecting the genome, but it is not established that increased levels of SCEs are predictive of cancer or any other health outcome. The one epidemiologic study on the question has not, to date, observed any effect of elevated SCEs on cancer, based on 6 years of follow-up and 49 observed cancers in a Nordic 6-year prospective study of 3,182 people (*25*). The increased levels of all translocations are somewhat more worrisome, as translocations are common in tumors and often occur at sites of genes affecting cell cycle and tumor suppression (*26*). Increased levels of chromosome breaks have been shown to be predictive of cancer in humans in the same Nordic study cited above (66 cancers observed) (*25*). Those in the highest tercile of aberrations had a twofold significant risk of cancer compared to the general population, while the lowest two terciles had no increased risk. A similar study in Italy based on 1,455 subjects (42 cancers) found an 80% increased cancer risk for those with medium or high levels of aberrations (*27*) after control for smoking and occupational exposures.

There is some potential for confounding of the genotoxic results by other genotoxic pesticides, specifically by another fungicide, captan. Captan has been found to be mutagenic in some animal and bacterial systems and has been found to cause cancer in some rodent studies (*28*). However, captan use has been uncommon in tomato application; owners relied primarily on EBDCs. The organophosphate pesticides commonly used with the EBDCs are not generally genotoxic.

In conclusion, our data are consistent with animal and some human data in suggesting a toxic effect of EBDCs on the thyroid and on the genome (SCEs, chromosomal translocations). However, the exposure effects observed in our study were subclinical and of uncertain future clinical significance. Furthermore, exposure effects were modest, generally on the borderline of conventional statistical significance, and were based on a single field study with limited sample size. The observed effects should therefore be interpreted with caution.

References

1. U.S. EPA. Ethylene bisdithiocarbamates (EBDCs); notice of intent to cancel and conclusion of special review. Fed Reg 57:7484–7530 (1992).
2. Jablonicka A, Plakova H, Harelova J, Vargova M. Analysis of chromosome aberrations and sister-chromatid exchanges in peripheral blood lymphoctyes of workers with occupational exposure to the mancozeb-containing fungicide Novozir Mn80. Mutat Res 224:143–146 (1989).
3. Smith D. Ethylene thiourea: thyroid function in two groups of exposed workers. Br J Ind Med 41:362–366 (1984).
4. Houeto P, Bindoula G, Hoffman JR. Ethylenebisdithiocarbamates and ethylenethiourea: possible human health hazards. Environ Health Perspect 103:568–573 (1995).
5. Kurttio P, Variainen T, Savolainen K. Environmental and biological monitoring of exposure to ethylenebisdithiocarbamate fungicides and ethylenethiourea. Br J Ind Med 47:203–206 (1990).
6. Prince J. Analysis of ethylenethiourea in urine by high-performance liquid chromatography. J Agric Food Chem 33:93–94 (1985).
7. Ramsey M, Moore D II, Briner J, Lee D, Olsen L, Senft J, Tucker J. The effects of age and lifestyle factors on the accumulation of cytogenetic damage as measured by chromosome painting. Mutat Res 338:95–106 (1995).
8. Perry P, Wolff S. New giemsa method for the differential staining of sister chromatids. Nature 251:156–158 (1974).
9. Minkler J, Stetka D, Carrano A. An ultraviolet light source for consistent differential staining of sister chromatids. Stain Technol 53:359–360 (1978).
10. Tucker J, Breneman J, Lee D, Ramsey M, Swiger R. Fluorescence *in situ* hybridization of human and mouse DNA probes to determine the chromosomal contents of cell lines and tumors. In: Cell Biology: A Laboratory Handbook (Celis JE, ed). Orlando, FL:Academic Press,1994;450–458.
11. Johnson G, Aroujio G. A simple method of reducing the fading of immunofluorescence during microscopy. J Immunol Methods 43:349–350 (1981).
12. Tucker JD, Morgan WF, Awa AA, Bauchinger M, Blakey D, Cornforth MN, Littlefield LG, Natarajan AT, Shasserre C. A proposed system for scoring structural aberrations detected by chromosome painting. Cytogenet Cell Genet 68:211–221 (1995).
13. Spruill M, Ramsey M, Swiger R, Nath J, Tucker J. The persistence of aberrations in mice induced by gamma radiation as measured by chromosome painting. Mutat Res 356:135–145 (1996).
14. Lucas J, Tenjin T, Straume T, Pinkel D, Moore D II, Litt M, Gray J. Rapid human chromosome aberration analysis using fluorescence *in situ* hybridization. Int J Radiat Biol 56:35–44 (1989).
15. Mendelsohn M, Mayall B, Bogart E, Moore D II, Perry B. DNA content and DNA-based centromeric index of the 24 human chromosomes. Science 179:1126–1129 (1973).
16. Tucker JD, Moore DH II. The importance of age and smoking in evaluating adverse cytogenetic effects of exposure to environmental agents. Environ Health Perspect 104(suppl 3):489–492 (1996).
17. SAS. SAS User's Guide: Statistics (Version 6.07). Cary, NC:SAS Institute, 1991.
18. Kurttio P, Savolainen K. Ethylenethiourea in air and urine as an indicator of exposure to ethylenebisdithiocarbamate fungicides. Scand J Work Environ Health 16:203–207 (1990).
19. Haskell D. Estimation of exposure of persons in California to pesticide products than contain mancozeb. Sacramento, CA:California Department of Pesticide Regulation, 1992.
20. Andreoli T, Carpenter C, Plum F, Smith L. Cecil Essentials of Medicine. Philadelphia, PA:Saunders, 1986.
21. Van Coevorden A, Laurent E, Decoster C, Kerkhofs M, Neve P, Van Cauter E, Mockel J. Decreased basal and stimulated thyrotropin secretion in healthy elderly men. J Clin Endocrinol Metab 69:177–185 (1989).
22. Mariotti S, Barbesino G, Caturegli P, Bartalena L, Sansoni P, Fagnoni F, Monti D, Fagiolo U, Franceschi C, Pinchera A. Complex alteration of thyroid function in healthy centenarians. J Clin Endocrin Metab 77:1130–1134 (1993).
23. Herschman J, Pekary A, Berg L, Solomon D, Sawin C. Serum thyrotropin and thyroid hormone levels in elderly and middle-aged euthyroid persons. J Am Geriatr Soc 41:823–828 (1993).
24. Bayer M. Effective laboratory evaluation of thyroid status. Med Clin North Am 75:1–26 (1991).
25. Hagmar L, Brogger A, Hansteen IL, Heim S, Hogstedt B, Knudsen L, Lambert B, Linnainmaa K, Mitelman F, Nordenson I. Cancer risk in humans predicted by increased levels of chromosomal aberrations in lymphocytes: Nordic study group on the health risk of chromosome damage. Cancer Res 54:2919–2922 (1994).
26. Rabbits T. Chromosomal translocations in human cancer, Nature 372:143-149 (1994).
27. Bonassi S, Abbondandolo A, Camurri L, Dal Pra L, De Ferrari M, Degrassi F, Forni A, Lamberti L, Lando C, Padovani P. Are chromosome aberrations in circulating lymphocytes predictive of future cancer onset in humans? Cancer Genet Cytogenet 79:133–135 (1995).
28. Hayes W, Laws E, eds. Handbook of Pesticide Toxicology. San Diego, CA:Academic Press, 1991.