Urine mutagenicity and lymphocyte DNA damage in fruit growers occupationally exposed to the fungicide captan

P Lebrailly, A Devaux, D Pottier, M De Meo, V Andre, I Baldi, F Severin, J Bernaud, B Durand, M Henry-Amar, P Gauduchon

Aims: To determine haematological parameters, urine mutagenicity (on three Salmonella typhimurium strains), and DNA damage (using the comet assay) in mononuclear leukocytes of farmers before and after a one-day spraying period of pear and apple trees with the fungicide captan in usual conditions.

Methods: Fruit growers were exposed to captan during the 1998 (n = 12) and/or the 2000 spraying seasons (n = 17). Biological samples were collected on the morning of the day of spraying (S1), the evening after spraying (S2), and the morning of the day after (S3). The UK Predictive Operator Exposure Model (UK-POEM) was used to quantify pesticide exposure intensity.

Results: No effect was observed on haematological parameters for these two spraying seasons. Proportions of mutagenic urine samples did not significantly differ between S1 and S2/S3 sampling points. In contrast, an inefficient protective clothing could correlate with an increase in urine mutagenicity as assessed by the TA102 tester strain.

Materials and Methods

Subjects
Selected individuals were identified through a local fruit grower organisation and lived in a single geographical area (département du Rhône) mainly devoted to orchards and vineyards (52% of farms). These subjects were enrolled because they currently used the fungicide captan on apple or pear trees. Informed consent was obtained from each subject prior to the beginning of the study. Information on individual characteristics (age, health status, and medical history) and lifestyle (diet, smoking habits including passive smoking exposure, alcohol, and medication) were collected as recommended, using a face to face questionnaire completed at the farmer’s home a few weeks/days prior to blood withdrawal and urine collection. Data on occupational exposure related to agricultural activities (pesticides used in crop treatment, chemicals used in cattle care, welding, mechanical repairs, etc) were collected at the S1 sampling point; details...
Main messages

- A one-day spraying period with captan and other pesticides does not significantly induce DNA damage in mononuclear leucocytes.
- Inefficient protective clothing could correlate with an increase in urine mutagenicity as assessed by the TA102 tester strain.

Pesticide exposure assessment

The United Kingdom Predictive Operator Exposure Model (UK-POEM) was used to calculate the predicted absorbed dose of captan. This model was developed by a panel of experts from data obtained after external pesticide exposure (UK-POEM) was used to calculate the predicted absorbed dose of captan. The predicted absorbed dose of pesticide is the sum of three components reflecting the absorbed dose from: (1) dermal exposure during mixing-loading tasks with a wettable sprayer being used. According to this model, dermal contamination, during mixing-loading tasks with a wettable powder (which is the formulation of captan used in this study) is estimated to be 100 mg for each bag of more than 1 kg. Estimated contamination during application is fixed to 400 ml and to 0.02 ml of diluted spray per hour of spraying for the dermal and respiratory routes, respectively. The fixed contamination is modulated by a number of variables for each step of pesticide use. For captan (wettable powder) on orchards, the following equations were used:

\[
\text{DEML} = 100 \text{ mg/bag} \times \text{NHS} \times \text{VCC/VP} \times \text{CAI/PC} \times \text{DA} \quad (1)
\]

Policy implications

- Assessment of occupational exposure needs to be improved and parameters predictive of intensity of exposure need to be validated.

where:

- NHS is the number of hectares sprayed
- VCC is the number of kg of commercial product used per ha
- VP is the weight of each bag (in kg)
- CAI is the concentration of the active ingredient in the formulation in mg/g (here 830 mg/g)
- PC equals 0.01 if rubber gloves are worn and 1 otherwise
- DA is the dermal absorption fixed to 0.1.

\[
\text{DEA} = \frac{(400 \text{ ml/h} \times \text{NHS} \times \text{VCC} \times \text{CAI} \times \text{VWH}) \times (\text{EH} + \text{EL} + \text{ET})}{\text{DA}} \quad (2)
\]

where:

- NH is the number of hours of spraying
- VWH is the number of litres of water per ha
- EH is the exposure of the hands that depends on of the use of rubber gloves (= 0.01) or not (= 0.025)
- EL is the exposure of legs that depends on the use of protective clothes (= 0) or normal clothes (= 0.013)
- ET is the exposure of the trunk that depends on the use of protective clothes (= 0) or normal clothes (= 0.0125)

\[
\text{REA} = 0.02 \text{ ml/h} \times \text{NHS} \times \text{VCC} \times \text{CAI} / \text{VWH} \quad (3)
\]

Cells

From peripheral blood samples, mononuclear cells were Ficoll separated and cryopreserved in liquid nitrogen without stimulation according to a previously described procedure.

Haematological parameters

Platelets, white cells (neutrophils, lymphocytes, monocytes, eosinophil and basophil granulocytes), and red cells counts were obtained using an automated counter S+(Coultronix).

Urine mutagenicity analysis

Eighty seven frozen urine samples were thawed at room temperature and filtered through a Whatman (No. 1) filter. A small volume was reserved for creatinine measurement by the Jaffé reaction, using a Cobas Mira+ device. Urine extraction was performed on pre-packed Bakerbond-spe-SDB-1 columns (Mallinkrodt Baker Laboratories, Noisy le Sec, France). Resins were first conditioned with 2×5 ml of methanol followed by 2×5 ml Milli-Q water. Urine samples were then washed with 10 ml Milli-Q water. This procedure eliminated traces of water soluble growth factors (especially histidine) from the resins. Residual water was removed by vacuum aspiration. Adsorbed substances were eluted with 10 ml of methanol/acetonitrile v/v. After evaporation to dryness with a SpeedVac system, residue was dissolved in dimethyl sulfoxide to reach a 500-fold concentration factor. Once prepared, urine extracts were kept in liquid nitrogen until use. All urine samples but one were subsequently concentrated. The microincubation procedure of the salmonella mutagenicity assay has been described by De Meo and colleagues. Mutagenicity was evaluated in overnight cultures of Salmonella typhimurium tester strains TA102 and TA97a without S9mix and YG1041 with S9mix. Each colony forming unit came from the growth of one bacterial cell that reverted its original mutation in the

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Viability and basal DNA damage in negative controls
Cell viability immediately after lymphocyte isolation was always above 98%. Immediately after thawing, viability of human lymphocytes was above 90%. After thawing, overall 10–25% of cells presented with DNA damage (damaged or highly damaged) in negative controls; the level of DNA damage did not significantly differ between experiments.

Statistical analysis
For haematological parameter changes, the difference between S1 and S3 was tested to null using the paired Student’s t test.
Results obtained with the Ames assay were analysed using two statistical tests in order to: (1) determine a dose-effect relation between the volume of urine extract and the number of His+ colonies using the models proposed by Kim and Margolin;17 and (2) detect a significant increase (mutagenic effect) or decrease (toxic effect) of at least one of the four doses of urine tested against the spontaneous background of His+ colonies. This analysis was made using the Dunnett test. Briefly, Kim and Margolin17 proposed a software (SALM) that performs regression analyses using one linear and two non-linear models.17 These arbitrary models tend to design typical dose-response curves that are obtained from pure chemicals. The two non-linear models include two parts: a dose-response increase, not necessarily linear; and a dose-response decrease related to toxicity. A sample was considered to be mutagenic when at least one of these two statistical tests provided a p value smaller than 0.05. The mutagenic power was then calculated as the difference between the highest number of His+ colonies at a given dose of urine extract and the spontaneous background of the corresponding experiment; it was expressed as the net number of revertants per mmol creatinine. The mutagenic power was fixed to null if none of the statistical tests described above were significant or if a significant toxic effect was detected (that is, number of His+ colonies smaller than the spontaneous background).
Parametric and non-parametric statistical tests were used for results obtained with the comet assay. The parametric paired Student’s t test was performed after exclusion of HDC. In results expressed using arithmetic means, changes in the proportion of HDC greatly modified the mean and could mask other DNA damage level modifications. Furthermore, distributions of DNA damage were not Gaussian. Finally, HDC might represent necrotic and/or apoptotic cells.18–20 The non-parametric Wilcoxon test was also used to compare changes in tail moment distributions between S1 and S3.

The relation between pesticide exposure and biological parameters was estimated using the χ² test or the Fisher exact test, as appropriate, or using a Spearman non-parametric regression model. The STATA statistical software (STATA Corporation, release 5.0), the SALM software, StatXact 4 for Windows (Cytel Software Corporation, 1998), and SAS release 6.12 software (SAS Institute, 1990) were used.

RESULTS
Overall, 19 farmers participated and provided biological samples for at least one of two spraying seasons. Urine and blood samples were collected for 12 farmers in the 1998 spraying season and for 17 during the 2000 spraying season. Viable lymphocytes, allowing for DNA damage assessment using the comet assay, were only available from the 1998 spraying season (12 S1 and 12 S3 blood samples); a total of 87 urine samples were collected (29 subjects with urine samples S1, S2, and S3).
All fruit growers were males, average age 39 years (range 22–53) for the 1998 spraying season and 40 years (range
of captan used per day of 4.9 kg (range 1.3–10.8). Fourteen per cent (n = 4) of the 29 days of mixing-loading of captan were without any protective clothing, 14% (n = 4) with mask or protective clothes, 17% (n = 5) with rubber gloves only, 14% (n = 4) with rubber gloves and mask or protective clothes, and 41% (n = 12) with all protective clothing (rubber gloves + mask + protective clothes). Twenty four per cent (n = 7) of application days were without protective clothing, 14% (n = 4) with protective clothes and/or mask, 14% (n = 4) with rubber gloves with protective clothes or mask, and 48% (n = 14) with all protective devices. Only four (14%) days of spraying activities were achieved with tractors equipped with an isolation cabin.

Using the UK-POEM, the mean predicted dose of absorbed captan was 14.8 mg (range 1.2–66.1) for the 1998 spraying season and 14.0 mg (range 0.9–45.3) for the 2000 spraying season (table 2). The mean body mass of fruit growers was 74 kg (range 59–85). The acceptable daily intake (ADI) for long term captan exposure in Europe is 0.01 mg/kg/day. Based on the predicted absorbed dose of captan calculated using the UK-POEM, all fruit growers presented with value above the ADI for chronic exposure with an average value of 19 times this ADI 0.19 mg/kg/day (range 1.06 to 82.6). No significant difference in heart rate and blood pressure were observed between S1 and S3. None of the haematological parameters significantly changed between the two blood sampling points except for a 20% significant (p<0.05) decrease in the number of basophils. No correlation was observed between haematological changes and pesticide exposure parameters (data not shown). Three farmers, however, had higher than normal values of eosinophils and two of them noted seasonal allergies.

Urine mutagenicity was evaluated on three Salmonella typhimurium strains: TA102 and TA97a without S9mix and YG1041 with addition of S9mix at three time points (S1, before exposure, compared to S2 and S3, after exposure). Table 4 indicates mean spontaneous frequencies of revertants. The day to day variability was low. Overall, 6% of urine samples (5/86) were mutagenic on TA97a without S9mix, 20% (17/86) on TA102 without S9mix, and 59% (50/85) on YG1041+S9mix. Even if the proportion of mutagenic urine samples was high at S1 for YG1041 (56%), none of the parameters related to confounding factors (medication, consumption of broiled meat the day before S1, smoking excepted) or to the type of work the day before S1 (pesticide exposure including pesticide spraying, re-entry tasks like pruning, tractor and sprayer maintenance, welding) were associated with that proportion or with the mutagenic power of urine samples (data not shown). Similarly, no relation was observed between the proportion of mutagenic samples or the mutagenic power on tester strains TA97a or TA102 and pesticide exposure the day before S1, type of pesticides handled (fig 2A) but not at S2. Such a relation (p = 0.03, Spearman's ρs = 0.40) positive correlation was observed between the predicted absorbed dose of captan and the mutagenic power of urine samples calculated for TA102 at S3 (fig 2A) but not at S2. Such a relation (p<0.05) was also found when the mutagenicity was quantified by the induced ratio (see table 4).

Three of the five fruit growers with a UK-POEM value exceeding 30 mg presented with significant urine mutagenicity at S3 on the TA102 strain versus only two (9%) of the 23 fruit growers with UK-POEM values below 30 mg (fig 2A). The five fruit growers with mutagenic urines on TA102 at S3 did not differ from the remaining 24 for other variables collected in the questionnaire (type of pesticides handled...
with captan, medication, etc). No statistically significant
differences in proportions of mutagenic urines were observed
according to the sampling points whatever the salmonella
strain. For the three strains, the mutagenic power of urine
samples was not statistically different between S1 and S2 or
S3. A positive correlation (p = 0.07 linear regression analysis,
Spearman’s $\rho_s = 0.56$, p = 0.002) was observed between the
difference (S3–S1) of the mutagenic power of urine samples
on the TA102 tester strain and the predicted absorbed dose of
captan (fig 2B). This relation could be a consequence of
protective clothing. It was the only parameter among those
used in the UK-POEM to calculate the predicted ingested
dose of captan (see methods section) to individually correlate
with the variation of mutagenic power on the TA102 tester
strain. An almost significant negative correlation
(Spearman’s $\rho_s = 0.37$, p = 0.051) was observed between
the protective clothing during mixing-loading tasks (see
table 2) and the variation of DNA damage level between
S1 and S3 (data not shown).

Lymphocyte DNA damage was measured for 12 farmers at
two sampling points (table 4). Overall, 10% of lymphocytes
were classified as damaged or highly damaged cells at S1
and 13% at S3. This level of DNA damage was lower or close to
those measured on negative controls for almost 70% of the
samples. No relations were observed between confounding
factors (alcohol or fruit beverage consumption, medication)
and level of DNA damage observed at S1 (data not shown).
No statistically significant variations of DNA damage were
observed between S1 and S3. Three of the 12 farmers,
however, showed a significant ($p < 0.01$, paired Student’s
$t$ test) increase in their mean level of DNA damage at S3; two
others exhibited only a significant ($p < 0.001$, Wilcoxon test)
increase in DNA damage level, and one a significant
($p < 0.001$, Wilcoxon test) decrease. No correlations were
observed between various pesticide exposure parameters
(table 2) and the variation of DNA damage level between
S1 and S3 (data not shown).

DISCUSSION
Captan has been shown to be genotoxic in numerous short
term assays in vitro.21 22 Captan is able to interact with DNA,
especially to form DNA adducts and DNA-protein cross-
links23 and more specifically inducing mutation through
base-pair substitution mechanisms including those detected
by TA102 or TA104 Salmonella typhimurium strains.24 25 Recent
studies have confirmed the ability of captan to be highly
mutagenic on strains detecting base-pair mutations,26 27 and
not on strains sensitive to frameshift mutations such as

<table>
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<tr>
<th>Table 1</th>
<th>Individual and lifestyle characteristics of the 19 fruit growers at enrolment in the study*</th>
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<tbody>
<tr>
<td>Mean age (SD) [range]</td>
<td>Smoking habits</td>
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<tr>
<td>38 (9) [20–53]</td>
<td>Current smokers</td>
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<td>*19 fruit growers for 29 different application days.</td>
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<td>†Antihistamine, antihypertensive, or anti-inflammatory drugs.</td>
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</table>
Genotoxic effect of occupational exposure to captan

TA98. Results from in vivo studies are even more dissimilar. Captan was not able to induce chromosomal aberration in the mouse. In an in vivo carcinogenicity bioassay, borderline results were obtained with captan on the induction of preneoplastic lesions in rat liver. Difficulties in studying in vivo captan genotoxicity might relate to its high cytotoxicity through mechanisms such as lipid peroxidation. Finally, captan belongs to IARC classification group III and to EPA classification group B2.

In the present study, urine mutagenicity (on three Salmonella typhimurium strains) and DNA damage (using the comet assay) in mononuclear leucocytes of farmers were measured before and after a one-day spraying period with captan on orchards in usual conditions. The analysis of the impact of pesticide exposure onto the biomarkers above was not only based on a comparison between before spraying (S1) and after spraying (S2 or S3) time points but also on the assessment of correlations between changes in biomarker levels and captan exposure related parameters. Moreover, the study was conducted for two different spraying seasons in order to assess the reproducibility of the observation. Urine mutagenicity and DNA damage measured by the comet assay on lymphocytes are suitable biomarkers in population studies when biological effects are examined shortly after exposure. As shown among fruit growers exposed to captan, urinary excretion of THPI, the main metabolite of captan, has been shown to reach its maximum level the day after exposure. Similarly, clastogenic activity in urine extracts obtained from fruit growers was detected within eight hours after pesticide application. These results suggest that time points for urine collections in the evening of the day of exposure (S2) and in the morning of the day after exposure (S3) are appropriate.

The protocol was designed to be the most sensitive for the detection of a mutagenic effect with the Ames assay, which led us to use an adsorbent appropriate to pesticide residues and a 500-fold concentration factor of urine. Moreover, the decision to confirm a mutagenic effect was mostly (79% of the 72 urines classified as mutagenic) based on the presence of a dose-response effect detected by the Kim and Margolin method.

Parameters used to quantify occupational exposure to pesticides in fields have most often been the area sprayed and/or the time spent in spraying (number of hours per day and/or number of days per year). Several studies have shown, at least for fruit growing, that other parameters could be of particular interest, such as the amount of pesticides handled and the type of spraying equipment. In a study conducted in the Netherlands on external dermal exposure in fruit growers applying captan on apple trees, the mean quantity of captan used in the observed days of exposure was 3.7 kg (range 0.3–10), which was very similar to that observed in our study. De Cock et al. measured levels of cutaneous contamination ranging from 0.47 to 48.6 mg/h/m², depending on pad location and type of spraying equipment.

Few models are currently available to simultaneously consider a set of quantitative (amount handled, duration of exposure, etc) as well as qualitative (protection used) exposure parameters. The UK-POEM is among those used for registration purposes in European countries. To our knowledge, it has not been used in studies focusing on genotoxic biomarkers. Based on the predicted absorbed dose of captan calculated with the UK-POEM, all fruit growers included in this study presented with a 19-fold increased exposure value on average (range 1.06–82.6) above the European acceptable daily intake (0.01 mg/kg/day). It would probably be more accurate to compare the UK-POEM calculated value to the acceptable operator exposure level (AOEL) derived from data issued from mutagenicity/carcinogenicity assays because only few days per year are devoted to captan spraying activities. However, these data are not presently available. Similarly, captan was the sole pesticide used on only 17% of the application days; on the remaining days up to five other pesticides were used. However, the amount of these pesticides represented only 14% of that of captan. Such a situation is usual in agricultural activities, especially in fruit growing, but also in open field farming. Our selection was targeted on captan because there was some evidence of its genotoxicity and it was one of the most used pesticides on orchards.

In our study, other pesticides were used the day before S1 in 45% of application days. These pesticides were almost identical to those used between S1 and S2/S3 (table 3). No relations were observed between biological parameters and pesticide used the day before S1. Since we did not observe any significant increases in urine mutagenicity or DNA damage after a one-day use of captan and other pesticides, we have no reason to believe that similar pesticides (captan excepted) will induce a genotoxic effect detectable at S1 and not at S2 or S3.

Our results show that the increase in the urine mutagenic power measured with strain TA102 correlated with the UK-POEM predicted value of absorbed captan. Moreover, this correlation was noted for the two spraying seasons. The correlation between UK-POEM values and urine mutagenicity on the TA102 strain could be a consequence of the influence of parameters related to protective clothing. These parameters are the only ones among all parameters included in the UK-POEM to correlate individually with the increase of the mutagenic power of urine samples.

In another study among non-smoking farmers with open field farming activities, the frequencies of mutagenic urine samples were 19% (7/37) on TA97a without S9mix and 25% (9/36) on TA102 without S9mix. In that study, no correlation

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<th>Table 3</th>
<th>Pesticides used the day before sampling and the day of captan use</th>
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<tr>
<td>Pesticide</td>
<td>Number of users (%)</td>
</tr>
<tr>
<td><strong>Fungicides</strong></td>
<td></td>
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<tr>
<td>Phthalimides</td>
<td>3 (10%)</td>
</tr>
<tr>
<td>Captan</td>
<td>1 (3%)</td>
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<tr>
<td>Folpet or iprodione</td>
<td>2 (7%)</td>
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<tr>
<td>Carbamates (thiophanatemethylderi, benomyl)</td>
<td>2 (7%)</td>
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<tr>
<td>Triazoles (fluazifolol, triadimenol, penconazole, myclobutanil)</td>
<td>1 (3%)</td>
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<tr>
<td>Phenols (dinocap)</td>
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<td>Dizaines (burjaminimethylo, chinomethionate, pyrazophos)</td>
<td>1 (3%)</td>
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<td>Thiadiazine (diazinomethylo)</td>
<td>-</td>
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<tr>
<td>Pyridine (pyrifenox)</td>
<td>1 (3%)</td>
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<tr>
<td>Sulphamide (flutifluonide)</td>
<td>-</td>
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<tr>
<td>Oxynilinolate</td>
<td>1 (3%)</td>
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<tr>
<td><strong>Insecticides</strong></td>
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<td>Oxamapholine (endosulphan)</td>
<td>2 (7%)</td>
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<tr>
<td>Carbamates (fenoxycarb, pyrimicarb, carbaryl)</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>Ureas (telflururon, flufenoxuron)</td>
<td>-</td>
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<tr>
<td>Orangophasor (phosalone, dimetfatoa, methidathion, vladophthy)</td>
<td>6 (21%)</td>
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<tr>
<td>Aveneim (abamectin)</td>
<td>-</td>
</tr>
<tr>
<td>Chloronicotin (imidaclopride)</td>
<td>2 (7%)</td>
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<tr>
<td>- Formamidin (amitrax)</td>
<td>-</td>
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<tr>
<td><strong>Herbicides</strong></td>
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<tr>
<td>Simazine, diuron, or glyphosate</td>
<td>2 (7%)</td>
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Active ingredients are italicised.
was observed between pesticide exposure and modifications of urine mutagenicity. The mutagenic frequencies calculated for the two strains were similar to those determined among fruit growers in the present study (6% and 20% for TA97 and TA102, respectively).

Although smoking is the major confounding factor in the evaluation of the mutagenic activity of urine samples using the Ames assay, other factors, such as alcohol consumption and medication, may play a role. In our study, a smoking effect was detectable on the YG1041 tester strain with the metabolic fraction only. The two smokers were among those presenting with the highest induced number of revertants. YG1041 is the resulting strain of *Salmonella typhimurium* TA98 in the plasmid carrying both acetyltransferase and nitroreductase genes was introduced. YG1041 expresses high nitroreductase genes was introduced. YG1041 expresses high

The percentages of damaged cells did not vary between the two sampling time points (10% versus 13%, for S1 and S3, respectively). These percentages were somewhat smaller than those measured previously in 41 farmers with open-field farming activities (16%, 14%, and 22% at the beginning, middle, and end of the spraying season, respectively) even after exclusion of current smokers. This higher level of DNA damage among open-field farmers could be a consequence of exposure characteristics such as the type of pesticides used or the lack of protective clothing in almost all farmers included in the study. Observed differences in DNA damage level between open-field farmers and fruit growers could also be due to other individual factors such as differences in food consumption.

Among confounding factors that could modulate DNA damage in healthy individuals, smoking and exhaustive physical activity could be ruled out in this study because none of the farmers were smokers in the 1998 spraying season, and subjects were their own controls, with blood sampling taken at rest on the mornings before and after pesticide exposure. Other studies have shown that highly damaged cells, also called hedgehog cells, represent necrotic and apoptotic cells. Excluding these cells from the analysis did not modify the results.

In conclusion, the lack of correlation between classical parameters of pesticide exposure and DNA damage level or urine mutagenicity can stem from three reasons: (1) a lack of genotoxic effect of captan in vivo in humans, at least at this level of exposure; (2) the existence of a genotoxic effect that would not modify the results of the tests used (that is, no genotoxic compound excreted in urine and no clastogenic compound present at a sufficient level in the blood); or (3) the used parameters are not accurate for estimating pesticide exposure. The positive correlation found between an integrated pesticide exposure parameter (UK-POEM value) and the mutagenic power onto the TA102 strain should be considered with caution even if it strengthens the need to increase the knowledge of parameters that should be used in the quantitative assessment of occupational exposure to pesticides. Studies are currently ongoing to assess the level of

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<th>Table 4 Results of urine mutagenicity using three salmonella tester strains and of DNA damage in lymphocytes</th>
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<td><strong>Genotoxicity assay</strong></td>
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<tr>
<td><em>Salmonella mutagenicity assay</em></td>
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<tr>
<td><em>Salmonella strain YG1041 with S9mix [MSB† = 143 (16)]</em></td>
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<td><em>Salmonella strain TA97 without S9mix [MSB† = 164 (15)]</em></td>
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<td><em>Salmonella strain TA102 without S9mix [MSB† = 337 (31)]</em></td>
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<td><strong>Alkaline comet assay</strong></td>
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*Time points for biological sampling: S1, the morning the day of capton use; S2, the evening the same day; and S3, the morning the day after.†MSB, mean spontaneous background (SD) between individual experiments.††Ratio between the highest number of reverse mutations in urine samples and the spontaneous background of the corresponding experiment.§Mutagenic power calculated as the net His colonies/mml urine creatinine for urines classified as mutagenic according to statistical analysis.√Percentage of nuclei ranked as damaged or highly damaged.††NT, not tested.
leucocyte DNA adducts in blood samples taken from the same population using the \(^{32}\)P post-labelling method.

**ACKNOWLEDGEMENTS**

We are indebted to Misses Bardou and Bruet for interviews with farmers, nurses who collected blood samples in the home, and Coope\'rative agricole SICOLY (in particular Mr Massardier) for the enrolment of farmers. Pierre Lebailly was recipient of a fellowship from the Ligue Nationale Contre le Cancer (Comite\’ de la Manche) which also granted this work (Comite\’s de la Manche et du Rh\’one), as well as the Union des Industries pour la Protection des Plantes, the Association pour la Recherche sur le Cancer, and the Conseil G\’en\’eral du Calvados.

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