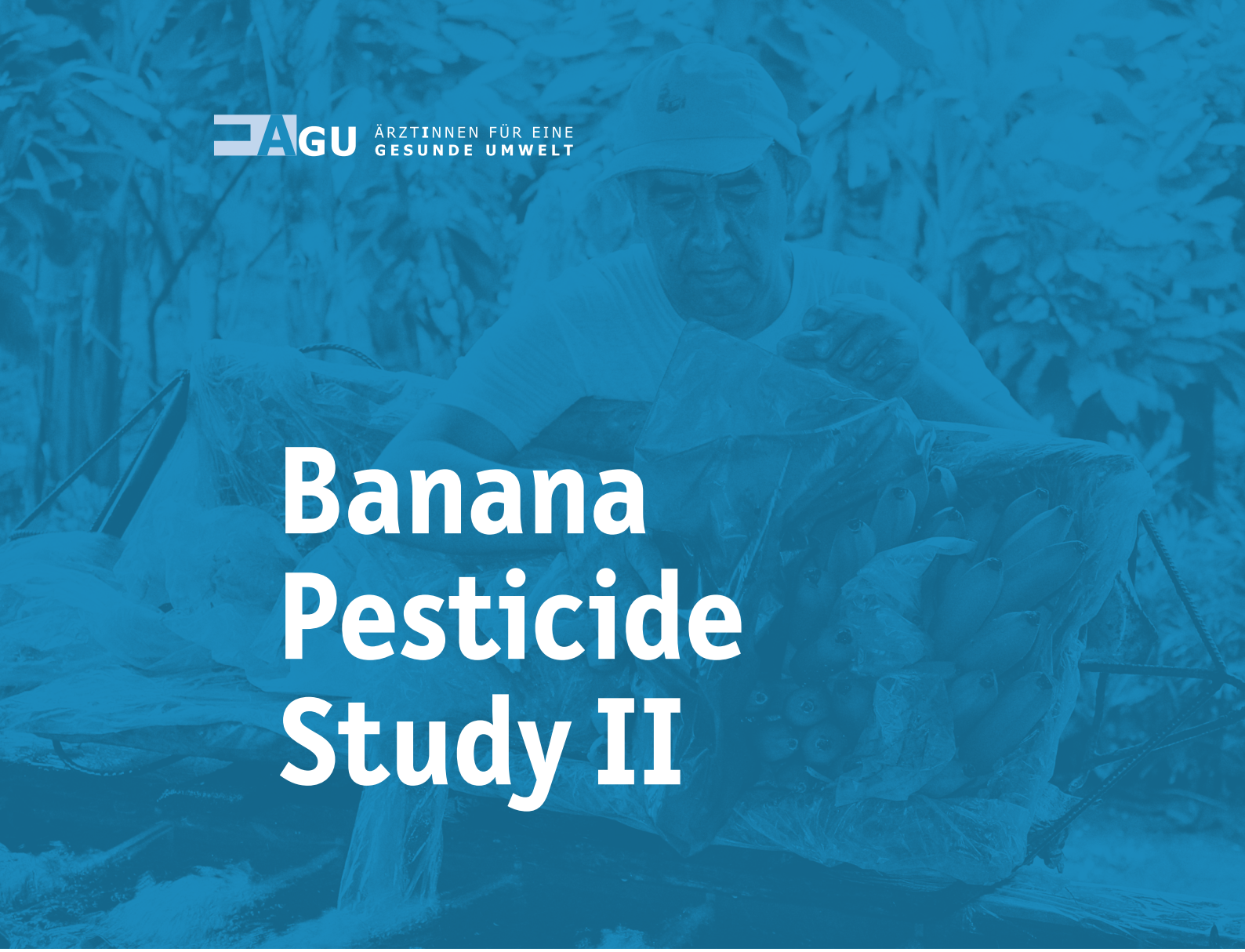

ÄRZTINNEN FÜR EINE GESUNDE UMWELT

Banana Pesticide Study II

Epidemiological study on small-scale farmers and farm workers in conventional and organic agriculture (Bananas) in Ecuador

Part 2:

Report on the Human Biomonitoring Study

September 18, 2016

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WIR DENKEN UMWELT.

Summary

Farm workers and small-scale farmers involved in conventional banana production are exposed to high levels of pesticides. In a study in Ecuador we examined the health status of pesticide users compared to other persons involved in banana cultivation who did not use pesticides.

The 71 participants in the study were first asked about any health symptoms experienced in the past six months. The results demonstrate significant differences between the two groups: Both local irritation symptoms (skin, upper respiratory tract, eyes) and gastrointestinal symptoms (nausea, vomiting, diarrhea) were considerably more frequent in pesticide users. This indicates that the use of pesticides is associated with acute adverse health effects in farm workers. For a detailed account see our Report on the Questionnaire Survey 2016 (www.aegu.net).

Now the results of the so called Micronucleus Assays are available as well. These tests involve first taking swabs of the buccal mucosa using wooden spatulas. Subsequently the cells are processed and scored for changes such as (additional) micronuclei, nuclear buds etc. Such nuclear anomalies are a first warning sign indicative of a carcinogenic potential. These cell anomalies were significantly more frequent in pesticide users than in the control group of non-pesticide users.

Our findings underline the urgent need for protection measures for the affected farm workers in banana plantations. The impact of pesticide use is not restricted to acute health effects, which are clearly more frequent in the exposed group. The results of the examinations of the buccal mucosa cells demonstrate impressively that the exposure to agrochemicals leads to long-term health risks as well. The results of our study suggest that pesticide users have a higher risk of developing cancer.

1. Performance of the Buccal Micronucleus Cytome Assay

Effect monitoring was carried out with the Buccal Micronucleus Cytome Assay. This non-invasive examination method is used for studying genotoxic and cytotoxic changes. The test is painless and involves no risks to the participants.

During the examinations, swabs of the buccal mucosa (from both cheeks separately) were taken with wooden spatulas (Tolbert et al. 1992). Immediately thereafter, the removed material i.e. the cells obtained were spread on the spot on one end of a microscope slide where a drop of sterilized water had been placed before with a sterile pipette.

The further processing of the cells and their very demanding evaluation was performed by experienced experts in an appropriate medical laboratory in Vienna after preparation according to the protocol of Thomas et al. (2009).

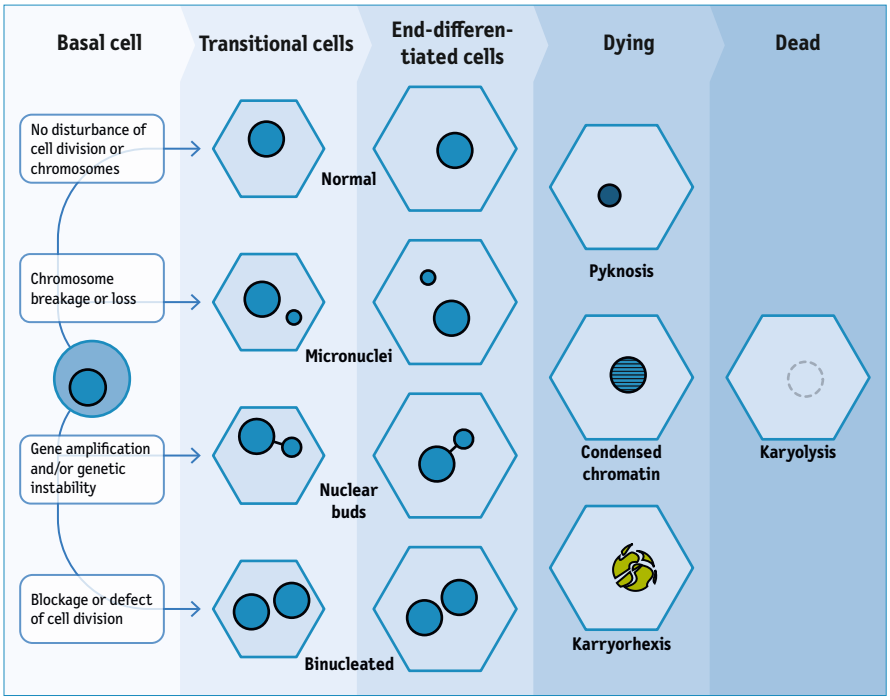
The cells on the microscope slides were stained with Feulgen (DNA-specific stain), washed under running water, rinsed for three minutes and then stained with Schiff's reagent at room temperature in the dark for 90 minutes. The slides were then counterstained with 0.2 % (w/v) Light Green.

After this DNA-specific staining and counterstaining, 1,000 differentiated cells and basal cells were first examined for deviations (micronuclei, nuclear buds, "broken eggs", binucleated cells, condensed chromatin, karyorrhexic, pyknotic and karyolytic cells) under a fluorescence microscope at 400-fold magnification; in a second step, an additional 1,000 differentiated cells were scored for micronuclei, nuclear buds and "broken eggs".

The term micronucleus refers to intracellular structures containing chromatin, wrapped in their own membrane and with no connection to the cell nucleus. They are formed during cell division by the exclusion of whole chromosomes (aneugenic effect) or chromatin fragments (clastogenic effect) from the cell nucleus as endpoint of genotoxicity. Other anomalies indicating genotoxic effects include nuclear buds (which result e.g. from gene amplification) and "broken eggs". Binucleated cells are indicative of cytotoxic effects, but also of a combination of genotoxic and cytotoxic effects. The various stages of cell death, which may have both genotoxic and cytotoxic causes, include Pyknosis, condensed chromatin (a more tightly packed chromatin structure), Karyorrhexis (higher condensation of the DNA's chromatin) and Karyolysis (dissolution of the cell nucleus, so called "ghost cell").

The various nuclear anomalies and their possible causes are shown in the overview below (Figure 1).

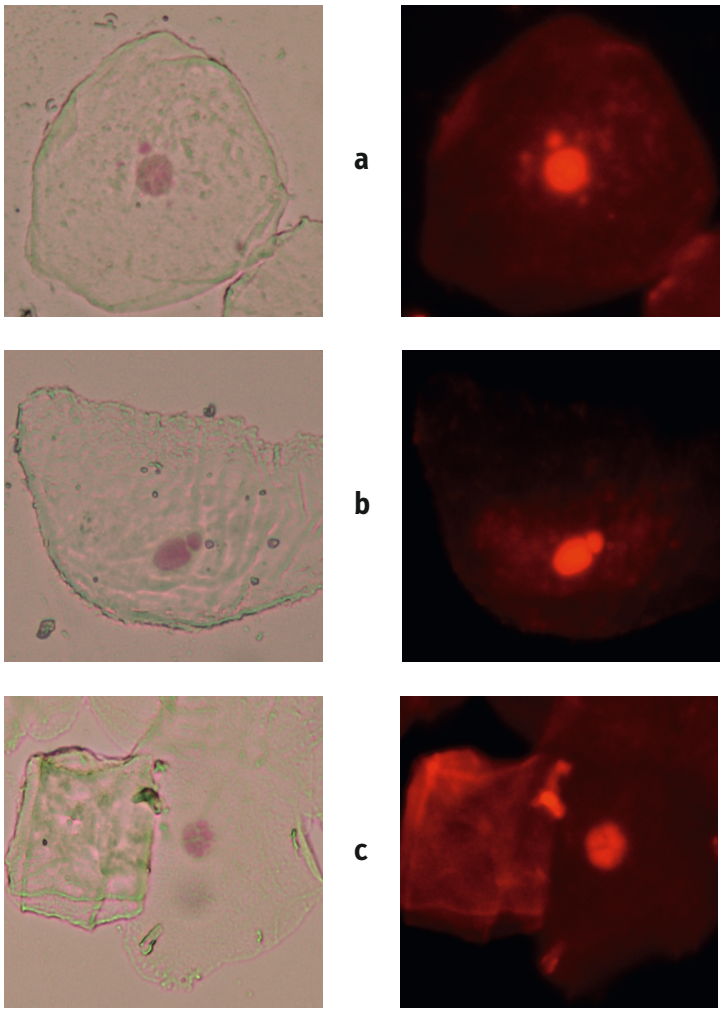
Figure 1
Development of differentiated buccal mucosa cells with and without the different types of genetic damage that can occur.



What such cells look like under the microscope with or without fluorescence filter is shown in Figure 2 (examples).

Figure 2
Examples of images of the different buccal cell types **a)** Cell with micronucleus, **b)** Cell with nuclear bud, **c)** karyorrhectic cell (right cell) and karyolytic cell (left cell).

DNA stained with Feulgen, cytoplasm stained with Light Green. Cells taken at 400-fold magnification under transmitted light (left images) and under fluorescence with a far red filter (right images).



2. Statistical method

The various cell anomalies were analysed by applying the general linear model, assuming poisson-distributed frequencies with a log-link. The analysis of differences of pesticides workers and controls were adjusted for age, education, smoking and alcohol consumption.

3. Results on genotoxicity and cytotoxicity endpoints

Endpoints indicating genotoxic effects include in particular micronuclei, nuclear buds, and "broken eggs". Endpoints which are rather indicative for cytotoxic effects are binucleated cells, condensed chromatin, Karyorrhexis, Karyolysis and Pyknosis. The results on these endpoints are shown in figure 3 below.

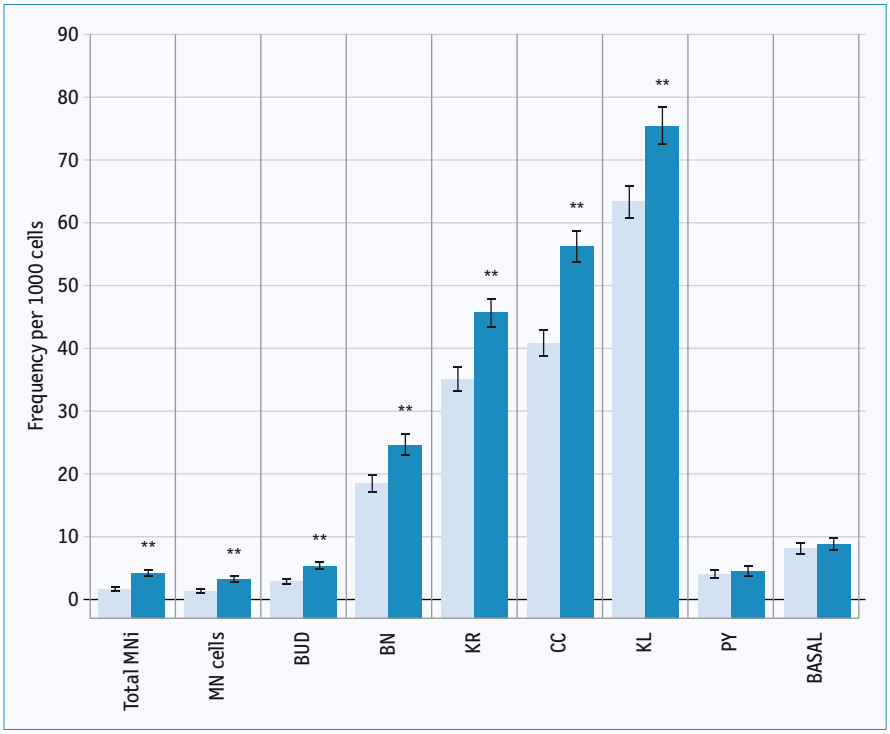


Figure 3
Nuclear anomalies in buccal mucosa cells of nonusers of non-pesticide (n=37, light bars) and pesticide users (n=31, dark bars). The y-axis indicates the frequencies of cellular anomalies per 1,000 cells. Bars and whiskers represent means and 95 % confidence intervals by non-pesticide users and pesticide users; **p<0.01;
Total MNi: Total number of micronuclei
MN cells: micronucleated cells (number of viewed cells with micronuclei)
BUD: Cells with nuclear buds („broken egg")
BN: Binucleated cells
KR: Karyorrhexis
CC: Condensed chromatin
KL: Karyolysis
PY: Pyknosis
BASAL: Basal cells.

The figure shows that the two groups (non-pesticide users: n=37; pesticide users: n=31) differ very strongly (highly significant) in regard to seven nuclear anomalies: The values of the parameters (nuclear anomalies) Total number of micronuclei and number of micronucleated cells, cells with nuclear buds, binucleated cells, karyorrhexis, condensed chromatin and karyolysis are considerably higher in pesticide users.

For example, the numbers of micronuclei, nuclear buds, binucleated cells and cells with condensed chromatin were 155, 84, 32 and 37 percent higher respectively in pesticide users than in non-pesticide users.

The frequencies of pyknotic and basal cells did not differ between the two groups.

4. Assessment of the results

The test performed is a well- established, standardized test for the detection of chromosomal aberrations. The present results demonstrate that the group of pesticide users exhibits significantly higher rates of nuclear anomalies than non-pesticide users.

Genotoxic anomalies are a first warning sign indicating a carcinogenic potential of the exposure. An increased rate of cell anomalies can thus be used to predict carcinogenic diseases.

Meanwhile, the results of various epidemiological studies on occupational pesticide exposure have been published. Though their findings are not always consistent, these studies still support the conclusion that farm workers exposed to pesticides might have a significantly higher risk of developing i.a. non-Hodgkin lymphomas and Leukemia (e.g. De Roos et al. 2003, McDuffie et al. 2001). Finally, these associations were also confirmed by the International Agency for Research on Cancer (IARC) for certain pesticides, e.g. for the herbicide glyphosate resp. its formulations (IARC 2015, Guyton 2015) which was often used by the participants in our study.

5. Overview of the results: Questionnaire and Micronucleus Assay

The participants were asked about any health symptoms experienced in the past six months. The results demonstrate significant differences between the two groups: Both local irritation symptoms (skin, upper respiratory tract, eyes) and gastrointestinal symptoms (nausea, vomiting, diarrhea) were considerably more frequent in pesticide users. This indicates that the use of pesticides is associated with acute adverse health effects in farm workers. For example, pesticide users had a 6 – 8-fold increased risk for reporting gastrointestinal symptoms (mostly nausea, vomiting, diarrhea) than non-users of pesticides (see our Report on the Questionnaire Survey 2016).

The results of the Micronucleus assays which are now available underline the urgent need for protection measures for the affected farm workers: The impact of pesticide use is not restricted to acute health effects, which are clearly more frequent in the exposed group. The results of the buccal mucosa cells assay demonstrate impressively that the exposure to agrochemicals leads to long-term health risks as well. The results suggest that pesticide users have a higher risk of developing cancer.

Overall we can conclude from our results that pesticide users do not only suffer from impaired well-being and impaired recovery during leisure time due to the symptoms, but are also at an increased risk of developing cancer.

6. References

De Roos AJ, Zahm SH, Cantor KP et al. (2003): Integrative assessment of multiple pesticides as risk factors for non-Hodgkin's lymphoma among men. Occup Environ Med 60:E11.

Guyton KZ, Loomis D, Grosse Y, El Ghissassi F, Benbrahim-Tallaa L, Guha N, Scoccianti C, Mattock H, Straif K (2015): International Agency for Research on Cancer Monograph Working Group, IARC, Lyon, France. Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate. Lancet Oncol 16:490–491.

Hutter H-P, Kundi M, Ludwig H, Moshammer H, Wallner P (2016): Bananen-Pestizidstudie: Epidemiologische Untersuchung von Kleinbauern und Landarbeitern im konventionellen und ökologischen Landbau (Bananen) in Ecuador. [Banana Pesticide Study: Epidemiological Survey of small-scale farmers and farm workers in conventional and organic agriculture (bananas) in Ecuador. Report on the Questionnaire Survey.] On behalf of ISDE Austria (International Society of Doctors for the Environment), Vienna, 2016. http://www.aegu.net/Bananen-Pestizidstudie.pdf

International Agency for Research on Cancer (IARC) (2015): Volume 112: Some organophosphate insecticides and herbicides: tetrachlorvinphos, parathion, malathion, diazinon and glyphosate.

IARC Working Group. Lyon; 3–10 March 2015. IARC Monogr Eval Carcinog Risk Chem Hum (in press).

McDuffie HH, Pahwa P, McLaughlin JR et al. (2001): Non-Hodgkin's lymphoma and specific pesticide exposures in men: cross-Canada study of pesticides and health. Cancer Epidemiol Biomarkers Prev 10:1155–1163.

Thomas P, Holland N, Bolognesi C, Kirsch-Volders M, Bonassi S, Zeiger E, Knasmüller S, Fenech M (2009): Buccal micronucleus cytome assay. Nat Protoc 4:825–37.

Tolbert P, Shy CM, Allen JW (1992): Micronucleus and other nuclear anomalies in buccal smears: Methods development. Mut Res 271:69–77.