Protocol for the project

Health effects of long term pyrethroid exposure on Bolivian pesticide applicators in public health vector control programmes

Overall objectives
The overall objectives of this project are to investigate the possible acute and chronic neurotoxicity of pyrethroid exposure on applicators working in public health vector control programmes.

Background
Residential pesticide spraying for vector control is an essential part in the prevention of malaria and other vector borne diseases (1;2) and the pesticide applicators are some of the most heavily pesticide exposed persons (3). In the public programmes in Bolivia two pyrethroids, lambda-cyhalotrin and deltamethrin have been almost the only pyrethroids used from the mid-90s, replacing the environmentally persistent organochlorates (i.e. DDT) and the acutely toxic organophosphates. Only in the last year a carbamate has been introduced in selected areas due to resistance among the insect vectors.

In Bolivia approximately 500 persons are employed by the Health Ministry as full time pesticide applicators in vector programmes. This vector programme is carried out as campaigns in the rainy season from November to May, and during this period the applicators are exposed 6 days a week, 8-10 hours per day. A series of complaints from the group of applicators have been reported in the form of acute and chronic symptoms after spraying, and the Ministry of Health in Bolivia has shown an interest in investigating the extent, severity, and possible cause of these complaints.

Since 2001 the Plagbol project, financed by DANIDA, has worked to limit the health effect of pesticides in humans and prevent pollution of the environment by teaching health personnel, technicians and farmers about personal protective equipment, hygiene, toxicity of pesticides, and first aid after intoxication. This project will be carried out in cooperation with the Plagbol project.

Health effects of pyrethroids
Pyrethroids are broad-spectrum insecticides which, during the last decades, have been widely used in agriculture, textile industry and in aircraft “disinfection”. The substances have been introduced in the public health vector programmes in residential environments and as the main active substance in insecticide-treated bed nets in the tropics. (2;4) Toxicologically, the pyrethroids are divided into two subtypes - type I exert their effects by postponing the inactivation of the sodium channels in the peripheral
and central nervous system just long enough to induce repetitive firing of action potentials - and type II
hold the sodium channels open so long that the membrane potential becomes depolarized to a point
where generation of action potentials might no longer be possible (5). Deltamethrin and lambda-
cyhalothrin are both type II. Other common pyrethroids are alpha-cypermethrin, cyfluthrin, permethrin,
and d-phenotrin. Pyrethroids enter the body mainly by inhalation and ingestion of dust and spray mist,
but also to some extent through absorption from the skin. Measurement of metabolites in urine has
been used in various studies to document the magnitude of acute exposure. A series of metabolites have
been documented, 3-phenoxybonzoic acid being a common metabolite of several pyrethroids (3;6).
Characteristically, the excretion of metabolites nearly ends within 24 hours after cessation of exposure,
so they can only be used to determine acute exposure.

As pyrethroids in mammals are rapidly detoxified in the blood and liver to inactive components and the
sodium channels are less sensitive to pyrethroids in mammals than in insects, the acute toxicity to hu-
mans is thought to be limited (7). Symptoms after acute intoxications are thought to be most common
in relation to type II pyrethroids (5). The most frequently reported symptoms after direct exposure are
paraesthesia of the eyes, face and breast, or symptoms from the respiratory tract, while systemic effects
like dizziness, nausea, palpitations, headache, anxiety, hyperactivity, ataxia, salivation, tremor, cho-
reoatetosis, clonic seizure and confusion are reported after ingestion or inhalation exposures (8-11).
However, the reports are descriptions of single or a few cases and do not reveal any information about
the dose response relationship or the actual frequency of the different health effects. Animal studies
show characteristic acute neurotoxic effects at high levels of ingestions but no significant effect of
dermal exposure at the same levels (12-14). The acute inhalation toxicity of pyrethroids in animal stud-
ies is also limited. (15;16)

The possibility of negative long lasting health effects of pyrethroids after exposure on the job, by acci-
dent or from suicidal attempts (4;17-19) has been widely discussed. In Germany the discussion of this
long-term, low-dose exposure in households and work environments has reached a point where control
operators refuse to use pyrethroids (20). A series of incidents have been recorded and the role of pesti-
cides, among these pyrethroids as risk factors for chronic debilitating neurological diseases as ALS,
Parkinson’s disease and Alzheimer’s disease, has been discussed, based mainly on single case reports
and case series (4;6;20-22). It has not been possible to elucidate the exact role of pyrethroids as most
patients in these case studies were also exposed to the considerably more neurotoxic organophosphates.
The effect of pesticides in general as risk factors for Parkinson’s disease is supported by some evidence
from epidemiological studies, while the relation between insecticides and other neurological diseases is
less well documented but of considerable importance for public health (23).
A new disease, ‘chronic pyrethroid-induced disease’, resembling the symptoms in chronic fatigue syndrome, sick building syndrome and Gulf War syndrome, has been claimed by some physicians but it is very controversial (19;24) An association with diabetes mellitus has also been demonstrated. (25) The critical effect of the type II pyrethroids is thought to be their neurotoxicity (12-14), but deltamethrin has also been shown to increase the risk of prostate cancer. (26) Possible reprotoxic effects are on neurodevelopment via the same mechanisms suspected to be responsible for possible neurotoxic effects (27).

The debate about pyrethroids in Europe and North America may have implications for their use in vector-borne disease control in developing countries, as attitudes to and policies concerning pesticides in the north have a strong influence on the use in the south. Properly designed neurobehavioral studies of groups with long-term exposure to pyrethroids do not exist (19;27). Therefore it is of great value to study the potentially negative chronic health effects of high-level pyrethroid exposure or single episodes of acute intoxication.

**Project objective**

- To describe the frequency, severity and persistence of health effects associated with acute high level pyrethroid exposure.
- To characterize the possible long term health effects as deterioration of neurophysiologic and neuropsychological performance in relation to long term exposure (duration and cumulative exposure) to pyrethroids.
- To establish a possible dose-response relation between short term and long term exposure and the occurrence of symptoms.

**Hypothesis**

- Symptoms of acute intoxications among pyrethroid applicators are reversible but can be related to the actual exposure during the spraying period.
- Possible chronic neurologic symptoms and objective decrease in performance can be detected among long term exposed pyrethroid applicators by comparing them with short term exposed and non-exposed control persons.
Study design

The study will be a cross sectional study of pesticide applicators and controls during a period with actual exposure to pesticides in the spraying season, combined with collection of information on historical exposure to pyrethroids (duration, intensity).

Materials and methods

300 persons are going to be included in the study, 150 male pesticide applicators from the public health vector programme and a control group of 150 not exposed males with the same socioeconomic characteristics. The participants will be randomly recruited from a pool of approximately 500 pesticide applicators and the controls from the not pesticide exposed inhabitants in the areas where the pesticide applicators live. Each person in the group of applicators and controls will be examined during the second half of the six-month spraying season on a weekday where the applicators have sprayed full time the day before.

Exclusion criteria will be exposure to other insecticides, especially organophosphates. Other criteria are age below 20 or above 60, psychiatric diseases, alcoholism or drug abuse, and for the controls former or current occupational exposure to pesticides or hospitalisation due to intoxications with pesticides.

Measured parameters

The clinical examinations will consist of a semi-structured interview revealing life-time pesticide exposure (intensity and duration), health status, symptoms (general and spraying-related), and family status. A general physical examination will include blood pressure, weight, height, lung- and heart function, inspection of the skin, and a standard neurological examination. To quantify a series of relevant neurological functions the Danish CATSYS tremor equipment will be included to measure reaction time, hand-arm coordination, balance (postural sway), and hand tremor (28).

A neurobehavioral test battery, including the tests from WHO’s core battery, will be used to evaluate cognitive and psychomotor function (29). These tests will be selected in cooperation with neuropsychological experts and implemented in versions which can be applied in rural areas on persons with limited language skills.

Exposure during spraying season will be self-reporting (by diary in selected periods) of daily spraying hours, the daily amount of pesticides used and personal protection used. As a biological measure of exposure blood and urine samples will be taken and analysed for metabolites of synthetic pyrethroids, especially 3-phenoxybenzoic acid (3).
Occurrence of specific symptoms in relation to spraying will also be collected by diaries in the spraying season.

**Perspectives**

Pyrethroids are of great importance, especially for vector control due to their presumably low toxicity to mammals compared to other pesticides. The debate about the possible long term toxicity of pyrethroids has serious implications for this use. There is, however, a serious lack of good studies as a basis for decisions about using pyrethroids.

The Bolivian pesticide applicators are a well-defined group with a long term high exposure to a limited number of pyrethroids and without significant exposure to more toxic organophosphates. Complaints about various health effects like trembling hands, altered motor coordination abilities, eye problems, skin irritation, change of character toward more irritability and intolerance have been reported, and the Bolivian Ministry of Health is interested in an examination of these workers.

To study this group and a control group would be an excellent way to evaluate the negative acute and chronic health effects of commonly used pyrethroids.

As the use of residential spraying and insecticide treated bed nets are essential tools to prevent vector borne diseases worldwide (1;2), a documentation of the possible health effects of pyrethroids on the most heavily exposed group would be an important contribution to our knowledge about the effects of pyrethroids, and it will have implications for the use in vector control programmes and in agriculture.

**Statistics**

For reasons of economical and temporal feasibility the number of subjects in the study will be limited to 300 (150 pesticide applicators and 150 controls). Previous studies on the neurological effects of carbamate and organophosphate pesticides on applicators have shown some significant effects with a similar number of subjects. (30-32)

The obtained data will be analysed with STATA. Univariate analyses will be performed using χ2 tests (categorical variables) and independent sample t-test or Kruskal-Wallis test (continuous variables). Multiple analyses will be performed using logistic regression for categorical outcome and linear regression for continuous outcome variables.

**Ethics**

The project was approved by the Medical Ethical Committee in Bolivia in 2008 and will be resubmitted for approval of the final study programme to be approved before start. The study will be in accordance
with the Helsinki Declaration and an informed consent will be signed by the participants before entering the study. Participation will be voluntary and should not pose any dangers or any nuisance to the persons entering the study.

Organisational support
Dr Erik Jørs has been the driving force in building research and educative networks in Bolivia for several years. He will be responsible for relations with the Bolivian Plagbol project – that will be the organisational framework for this study. An agreement has been signed between the Plagbol Foundation, the Bolivian Ministry of Health (Programme for Vector Borne Diseases) and the University of La Paz. Each of these institutions will appoint a person to participate in the planning and execution of the project.

Presentation
The findings of the projects are to be published in an international peer-reviewed journal. The sequence of authors is to be as follows: Martin Rune Hansen, Flemming Lander, Vivi Schlünssen, Ariadne Condorco and Erik Jørs.

Schedule

01.02.2012-31.03.2012: Preparations including planning of field work.
01.07.2012-31.08.2012: Data entry; clean-up of data.
01.11.2012-31.01.2013: Preparation of report and article.

Qualifications of the stipendiate
As can be seen from the CV attached to this application, the stipendiate Martin Rune Hansen is currently studying for his Master of Science in medicine, and the average of his grades from Aarhus University at the time of application is 11.8 on the Danish 7-stage grade scale. He has previously travelled and worked as a volunteer in developing countries for extended periods, meaning he has a good understanding of what it means to work in developing countries. This is crucial, as the project involves three
months field work in Bolivia. Furthermore, his bachelor report assessed the toxicity of DDT, another pesticide used for vector control.
References

(1) WHO. Indoor residual spraying - Use of indoor residual spraying for scaling up global malaria control and elimination. WHO/HTM/MAL/2006.1112
(2) WHO. The technical basis for coordinated action against insecticide resistance: preserving the effectiveness of modern malaria vector control. 2011.
(15) Junshi Miyamoto. Degradation, Metabolism and Toxicity of Synthetic Pyrethroids. Environmental Health Perspectives 1976;14;15–28
(16) Berteau PE, Deen WA. A Comparison of Oral and Inhalation Toxicities of Four Insecticides to Mice and Rats. Bull Environ Contam Toxicol. 1978 Jan;19(1);113-20


