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Biological indicators for the assessment of human exposure to industrial chemicals

Report

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Biological indicators for the assessment of human exposure to industrial chemicals

Alkyl lead compounds
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Preface of the first volume

The evaluation of the exposure of workers to dangerous agents is one of the measures insuring a better health protection. This evaluation is called monitoring.

Two approaches are available for the monitoring :
- ***ambient monitoring*** already in use for many years and
- ***biological monitoring*** of more recent development.

The need for clear definitions and for establishing the respective roles of these two types of monitoring has become necessary recently. In 1980 in Luxembourg at an international seminar organized jointly by the CEC and the United States authorities (Occupational Safety and Health Administration and the National Institute for Occupational Safety and Health) on the Assessment of Toxic Agents at the Workplace, the following definitions were agreed :

- ***ambient monitoring*** is the measurement and assessment of agents at the workplace and evaluates ambient exposure and health risk compared to an appropriate reference;
- ***biological monitoring*** is the measurement and assessment of workplace agents or their metabolites either in tissues, secreta, excreta, expired air or any combination of these to evaluate exposure and health risk compared to an appropriate reference.

In addition, the term "Health Surveillance" was also defined as the periodic medico-physiological examinations of exposed workers with the objective of protecting health and preventing occupational related disease. The detection of established disease is outside the scope of this definition.

The definitions of biological monitoring and health surveillance separate components of a continuum which can range from the measurements of agents in the body through measurements of metabolites, to signs of early disease. A problem left unresolved concerns the precise place within these definitions of certain biochemical tests such as zinc protoporphyrin (ZPP), delta aminolaevulinic acid dehydrase (ALA-D), delta aminolaevulinic acid (ALA) in the blood and urine, etc., which are, in fact, indicators of metabolic effects which have occurred as a consequence of exposure.

Ambient monitoring is carried out for different reasons, for example :

a. determining ambient concentrations in relation to an established legal standard or consensus guideline;
b. determining the relationship, if any, between the concentrations of agents at the workplace and the health of the workers;
c. ensuring the effectiveness of control measures;
d. evaluating the need for controls in the vicinity of specific emission sources;
e. indicating trends in relation to an improvement or determination at the workplace;
f. providing an historical record.

Biological monitoring measures or evaluates exposure from all routes. It sometimes allows a better evaluation of health risk than ambient monitoring especially in cases where exposure through different routes has to be considered.
Biological monitoring takes into account individual variability, the impact of factors such as personal activity, biological characteristics and life styles of the individual.

The two types of monitoring are complementary in increasing the protection of workers' health. If both are carried out simultaneously, information should be produced on the relationships existing between external exposure and concentration of the substance in biological samples, and between this concentration and early effects.

Detailed knowledge of the metabolism of the toxic agent in the human organism and of the alterations that occur in the critical organ is essential in selecting the parameter to be used as indicator.

Unfortunately, however, such knowledge is usually insufficient and thus limitations exist in most biological monitoring programmes.

The conditions necessary for successful biological monitoring are :

- existence of indicators,
- existence of analytical methods that will guarantee technical reliability in the use of these indicators,
- possibility of measuring the indicators on readily accessible biological specimens,
- existence and knowledge of dose-effect and dose-response relationships.

In carrying out a biological monitoring programme, it is indispensable to know exactly what the characteristics and behaviour of the indicators under study are in relationship to length of exposure, time elapsed since beginning and end of exposure, and all physiological and pathological factors other than exposure that could give a false interpretation of the results obtained.

Conditions for biological monitoring application include adoption of analytical methods yielding values comparable throughout the different laboratories.

This long time adopted approach has already permitted the CEC to standardize in 1972 a method for erythrocyte ALAD determination and develop programmes for inter-laboratory comparisons for lead and cadmium determination in biological media.

The Council of Ministers of the European Communities in adopting in 1978 the First Action Programme on Safety and Health at Work proposed by the Commission stressed the need to increase protection against dangerous substances; it emphasized the need to promote new monitoring and measuring methods for the assessment of individual exposure, in particular through the application of sensitive biological indicators.

In August 1982 the Council adopted a directive on the protection of workers exposed to lead. The monitoring of blood lead levels as well as the determination of ALAU, ALAD and ZPP are among the tools to be used for monitoring worker exposure to lead. A comparison of the results with action levels and limit values allows appropriate action to be taken.

Considerable data concerning the biological monitoring of a number of industrial chemicals has been published in the international literature.

Nevertheless, the difference in approaches used in the research, the variety of analytical methods and the frequent discordances in the results, usually make it difficult to formulate a conclusive synthesis permitting the transfer of literature data into practice.

The aim of this series dedicated to human biological monitoring of industrial chemicals in occupational health is based on the considerable experience acquired by the authors in the specific topics.

For the draft of the monographs, the following outline, suggested by R.L. Zielhuis and R. Lauwerys, has been used :

- a review of metabolism and/or mechanism of action;
- potentially useful biological parameters for evaluation of exposure and/or body burden and/or early reversible effects;
- a critical evaluation of each parameter :
  - predictive validity in regard to exposure;
  - quantitative relationship between levels of external exposure and internal exposure, and between exposure and effects;
  - limitations of the test;
- a proposal for one or several tests for biological monitoring.

Because of the considerable gaps in scientific knowledge it has not been possible to

follow this outline strictly in every single one of the monographies. It is hoped that future research will fill these gaps.

It must be recognized that the biological monitoring approach for other toxic agents must still be developed and that considerable research is still necessary.

The Council in the above mentioned action programme and in the directives recently adopted in this field stressed the need to provide adequate information at all levels. It is considered that these monographs will be of benefit to the occupational health physicians, the industrial hygienists, the employers and the trade-union representatives, by giving the scientific rationale on which a number of biological monitoring programmes are based.

The Editors
1983

Preface of the second volume

Last year we published a series of monographs in one volume under the title "Human biological monitoring of industrial chemicals series" in which Benzene, Cadmium Chlorinated Bydrocarbon solvents, Lead, Manganese, Titanium and Toluene were discussed.

When preparing these documents each author was asked to pay particular attention to the problem of the quantitative relationships between the levels of external and internal exposure and between exposure and effects.

In a number of cases information on the levels of biological indicators which are indicative of current exposure without short term detectable health effects is available.

However we were already confronted with the impossibility to determine at present if the levels for biological indicators without short term detectable health effects can also be considered as adequate with respect to longer term effects.

In preparing the present series of monographs it became apparent that for a number of biological indicators corresponding to biochemical tests, it is not yet possible to establish if these are indicative of early reversible effects and would thus qualify for terminology of "biological monitoring" as defined in the preface of the first volume. For many substances, extensily used in industry, biological indicators are being developed but still require extensive assessment before possible routine application.

As the object of these monographs is to provide up to date scientific information not only for the chemical substances for which biological indicators could be rised routine, but also for the many more substances for which biological indicators are at the early stage of development it was considered advisable to change the title of the series to **"Biological indicators for the assessment of human exposure to industrial chemicals".**

We hope that this new title will avoid giving the impression to the reader that for all the substances presented in this volume and the substances ones, biological indicators can be already routinely applied.

The Editors
1984

Preface of the third volume

Following the established yearly frequency, we are happy to present the third volume in the series of monographs on "Biological Indicators for the Assessment of Human Exposure to Industrial Chemicals", which are addressed to occupational health physicians, industrial hygienists and, in general, to all who are concerned with prevention in the workplace.

The original title of the first volume of the series "Human Biological Monitoring of Industrial Chemicals Series" was changed in the second volume and this change is now further justified by the four monographs making up the third volume : alkyl lead compounds, dimethylformamide, mercury and organophosphorus pesticides.

As in the previous volumes, the scope of the publication has not been limited to the most widely known and used toxic industrial agents. It was felt that consideration should also be given to other substances, where recent scientific advances have suggested the need to verify how far assessment of exposure using biological indicators is reliable in real industrial situations. One of the aims of this series is, in fact, to stimulate further research, especially applied research, that would have the task of validating, on large groups of workers, preliminary scientific observations that are usually obtained from studies on relatively small groups of subjects and often in controlled experimental exposure situations.

Eighteen monographs have now appeared in this series published by the Commission of the European Communities. The previous two volumes covered fourteen monographs on acrylonitrile, aluminium, benzene, cadmium, chlorinated hydrocarbon solvents, chromium, copper, lead, manganese, styrene, titanium, toluene, xylene, zinc.

Highly competent scientists from the following European scientific and research institutes have contributed in preparing the monographs : Cattedra di Medicina del Lavoro dell'Università di Parma (Italy), Clinica del Lavoro "L. Devoto" dell'Università di Milano (Italy), Coronel Laboratorium, Universiteit van Amsterdam (the Netherlands), Institut für Arbeits- und Sozialmedizin der Universität Erlangen-Nürnberg (F.R. Germany), Unité de Toxicologie Industrielle et Médicale, Université de Louvain, Bruxelles (Belgium).

It is planned in the future to extend cooperation to other scientific institutes and thus involve a wider number of scientists and experts.

The fourth volume, which is already under way, will include monographs on "Aldrin, Dieldrin and Endrin" by N.J. Van Sittert, Shell Internationale Petroleum (the Netherlands); "Non-Substituted Aliphatic Hydrocarbons" by K.H. Cohz, Danish National Institute of Occupational Health, Hellerup (Denmark); "Arsenic" by V. Foà, Clinica del Lavoro, University of Milan, (Italy); "Vanadium" by K.- H. Schaller, Institut für Arbeits- und Sozialmedizin, University of Erlangen-Nürnberg (F.R. Germany).

The Editors
1986

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Alkyl Lead Compounds

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Summary

The main route of absorption of alkyl lead compounds is via the respiratory tract but the skin, too, offers an excellent penetration route for both tetraethyl lead (TEL) and tetramethyl led (TEM). The toxic effects of alkyl lead compounds involve mainly the central nervous system. Only slight alterations in heme synthesis have been demonstrated, which contrasts with the situation in the case of inorganic lead.
Lead in urine (PbU) is at present the only biological indicator that can be used for assessment of occupational exposure to organic lead compounds.

Abbreviations

TEL	Tetraethyl lead
TEM	Tetramethyl lead
PbB	Lead in blood
PbU	Urinary lead
PbU-EDTA	Amount of chelatable lead excreted with 24-h urine after administration of $CaNa_2EDTA$
ALAD	Delta-aminolevulinic acid dehydratase activity of erythrocytes
EP	Erythrocyte protoporphyrin
ALAU	Delta-aminolevulinic acid in urine
CPU	Urinary coproporphyrin

Alkyl Lead Compounds

Introduction

Alkyl lead compounds must be discussed separately from inorganic lead because of the quite different mode of absorption, metabolic transformation and pathological pattern which involve the central nervous system in particular. Even though a large number of alkyl lead compounds exists only tetraethyl lead (TEL) and tetramethyl lead (TEM) are of practical importance in industry where, due to their exceptional antiknock properties, they are used as additive in petrol for internal combustion engines to increase the "octane number". The concentration of alkyl lead compounds in petrol varies according to the regulations in force in different countries: an EEC Directive requires that Member States impose a maximum lead limit of 0.15 to 0.40 g/l (1978).
Alkyl lead compounds used as additive in petrol constitute one of the largest sources of inorganic lead pollution of the air, especially in urban areas. The quantity of organic lead compounds emitted with combustion fumes does, however, account for only a small part of the metal contained in the petrol (between 0.7% and 1.1%) (Grandjean and Nielsen, 1979). Therefore the problem of environmental pollution due to the use of alkyl lead compounds as antiknock agents seems to be mostly the result of emission of inorganic lead with motor vehicle exhaust. However, a study by Nielsen et al. (1978) revealed significantly higher encephalic concentrations of trialkyl lead compounds in subjects not occupationally exposed to alkyl lead compounds living in urban areas, compared with the encephalic concentrations detected in subjects living in rural areas.

Occupational exposure may occur during the production, transport and mixing of TEL and TEM with gasoline, but only a relatively small number of cases of poisoning has been reported (Grandjean and Nielsen, 1979). Hazardous situations may occur during the cleaning of leaded gasoline storage tanks (Kitzmiller et al., 1954; Akatsuka, 1973). Other subjects particularly exposed are filling stations attendants, staff in garages and underground parking lots and motor mechanics who use gasoline to clean their hands (Leavitt, 1934; Gutniak et al., 1964).

Gasoline sniffing has been the cause of several cases of alkyl lead compounds poisoning diagnosed among children (Grandjean and Nielsen, 1979).

Physico-Chemical Properties

Tetraethyl lead

Formula	$Pb(C_2H_5)_4$
Constants	Molecular weight: 325 MP: —136.8°C BP: 198 to 202°C with decomposition D: 1.659 at 18°C vapour pressure : 1 mm Hg at 38.4°C

Colourless, oily liquid, pleasant characteristic odour, soluble in benzene, ethyl alcohol and ethyl ether.

Tetramethyl lead

Formula	$Pb(CH_3)_4$
Constants	Molecular weight: 267.33 MP: —27.5°C BP: 110°C D: 1.99 Vapour pressure: 22 mm Hg at 25°C

Colourless liquid, slightly soluble in benzene, ethyl alcohol and ethyl ether.

Effects on Humans

The toxic effects of alkyl lead compounds involve the central nervous system.
Absorption of high quantities of TEL over a short period or of lower quantities over prolonged periods gives rise to a pattern of intoxication affecting the nervous system. In the less severe forms, the characteristic clinical symptoms are loss of appetite, nausea, metallic taste in the mouth, troubled memory and sleep (Hamilton et al., 1925; Sanders, 1964; Akatsuka, 1973). As the intoxication progresses, other clinical symptoms appear (Machle, 1933) : the patient undergoes profound character changes, with recurrent episodes of complete disorientation, hallucinations, facial muscle contraction, muscular hyperactivity and increased resistance to physical fatigue. These episodes may suddenly change to maniacal symptoms similar to "delirium tremens" or to violent convulsions that may lead to coma or death.

The period between the cessation of exposure and appearance of the initial symptoms may vary from a few hours in the most severe, rapidly evolving cases, to 8-10 days in mild cases (Kehoe, 1983).
The neurological changes that occur during TEL intoxication appear to be in the encephalus. Some authors suggest that triethyl lead (the toxic metabolite of TEL) acts by inhibiting cell respiration, probably through uncoupling of oxidative phosphorylation (Cremer, 1959; Cremer et al., 1961; Nielsen et al., 1978).

According to Galzigna et al. (1975) "the action of alkyl lead derivatives of lead can be explained by their multimodal effects on the CNS and particularly by the interference with cholinergic, adrenergic and serotonine-ergic processes. The toxic action of triethyl lead might be related mainly to a transformation of norepinephrine into an aminochrome which is stabilized by acetylcholine leaking from cholinergic neurones after inhibition of pseudo-cholinesterase. The combined effects result in the formation of a psychotogenic complex particularly active at the central nervous system level".

According to a more recent hypothesis trialkyl lead compounds elicit increased neurone excitability probably because of a changed distribution of the Cl^- ions via the cell membranes (Cremer, 1984). The increased neurone activity gives rise to an increased glucose utilization and consequently an accumulation of piruvic and lactic acids. In addition, TEL and TEM may have a direct effect on the mitochondria, inhibiting the entry of substrates (Skilleter, 1975) and ATP synthesis.
The neurotoxic action of diethyl lead seems to be far less marked than that of trialkyl compounds.

TEM intoxication appears to resemble TEL intoxication, even though it is usually less severe (Sprigman et al., 1973; Gething, 1975).
Up to 1979 about 100 cases of fatal TEL intoxication had been reported but no fatal cases of TEM intoxication appear to have occurred (Grandjean et al., 1979).

As regards other clinical effects, contrary to the situation with inorganic lead, during intoxication by alkyl lead compounds no evidence of significant alterations in blood chemistry, abdominal colic or peripheral neuropathy has been recorded.

Finally, it has been recently suggested, on an experimental basis, that alkyl lead compounds may exert a genotoxic effect. Experiments performed on ***Drosophila Melanogaster*** have in fact shown an increased incidence of non-disjunction and chromatid breaks (Ahlberg et al., 1972; Ramel, 1973).
A study made on human lymphocytes treated in vitro with trialkyl lead compounds (the toxic metabolites of tetra alkyl lead compounds) demonstrated cytogenetic effects, such as an increase in sister chromatid exchanges (SCE) and reduced chromosome length, at concentrations of the toxic in the culture similar to those found in blood and tissues of exposed subjects (1 μmol/l) (Grandjean and Andersen, 1982).

On the question of a possible human carcinogenic effect, the negative result of the only mortality study undertaken on workers exposed to TEL and observed for 20 years

(Robinson, 1974 b) does not permit any definite conclusions to be drawn, since the population in this study was of a relatively young age (mean age 31.5 years, range 20-58 years).

Furthermore, in Robinson's studies (1974 b, 1976) the mortality and morbidity figures for workers exposed to TEL are the same as in unexposed workers; the author examined two groups of subjects employed in the production of TEL for more than 20 years whose exposure was "within a range termed 'safe' by current industrial medicine standards".

Metabolism

Absorption

In working environments TEL and TEM are easily absorbed via the respiratory tract but, due to their high liposolubility they are also easily absorbed via the skin. TEM is absorbed less rapidly than TEL via this route (Kehoe, 1983). The digestive system is not a major route of absorption in the working environment.

Distribution

In blood, lead was found predominantly in the lipid fraction obtained by extraction with chloroform-methanol : the mean proportion of lead extracted in blood lipids in three patients with TEL poisoning was 73.1%, which was three times as high as the proportion in blood lipids in normal subjects (24%) and in subjects with inorganic lead poisoning (24%) (Beattie et al., 1972).

The alkyl compounds are transported from the blood to the liver where they undergo metabolic degradation by endoplasmic reticulum enzymes (Cremer, 1959). A small proportion is also metabolized in the kidney and encephalus, while probably no significant metabolization takes place in the blood, spleen and muscles (Bolanowska et al., 1971). Cremer (1959) demonstrated experimentally that TEL is degraded to triethyl lead, diethyl lead and metallic lead according to the following reaction:

$$(CH_3CH_2)_4Pb \rightarrow (CH_3CH_2)_3Pb^+ \rightarrow (CH_3CH_2)_2Pb^{++} \rightarrow Pb^{++}$$

The conversion of TEL to trialkyl compounds is relatively rapid, but conversion to diethyl lead and Pb^{++} is slow. The formation of diethyl lead was not confirmed by Bolanowska (1968) in experimental animals, but appears to have been demonstrated in man (Chiesura, 1970; Yamamura et al., 1982).

High quantities of triethyl lead concentrate in the brain, reaching much higher levels than TEL. High amounts are also found in blood and liver. Contrary to inorganic lead triethyl lead does not have a particular affinity for the bone tissue.

The metabolic transformations by TEM are probably similar to those described for TEL, since TEM is transformed to a trialkyl compound, i.e., trimethyl lead.

The lower toxicity of TEM compared to TEL appears to be due to slower absorption and slower metabolic transformation, with a consequently lower concentration of neurotoxic metabolites in the central nervous system.

Excretion

The degradation products of TEL and TEM are mainly eliminated with the urine. It has also been demonstrated that tetraalkyl compounds can be partially eliminated with expired air (Heard et al., 1979). The portion excreted with the feces is mostly due to the high biliary excretion of organic lead compounds (Kehoe, 1931). Excretion via the hair, perspiration and maternal milk appears to be minimal (Jensen, 1984).

Biological Indicators

The indicators of internal dose (PbB, PbU, PbU-EDTA) and of effect (ALAD, EP, ALAU, CPU) that are commonly used for biological monitoring in occupational exposure to inorganic lead, behave very differently following exposure to alkyl lead compounds.

Blood lead levels are generally not significantly high and rarely exceed 50 μg/100 ml (Muller, 1953; Kehoe, 1964). In workers exposed daily to low concentrations of TEL or TEM, the mean lead concentration in blood was 41-45 μg/100 ml (De Treville et al., 1962; Robinson, 1976).

In the subjects studied by Kitzmiller et al. (1954) the PbB levels were below 60-70 μg/100 ml even in the presence of severe intoxication. Lead levels around 400 μg/100 ml were found in cases of fatal human poisoning (Bolanowska et al., 1967; Mizoi et al., 1973).

In subjects exposed to alkyl lead compounds blood lead binds mainly with the lipid components: the mean percentage of lead in the lipid fraction of whole blood was about three times higher in the subjects exposed to alkyl lead compounds compared to "normal subjects" or subjects with metallic lead poisoning (Beattie et al., 1972). The correlation between severity of symptoms and lipid fraction lead levels seems to be closer than the correlation between symptoms and total blood lead levels.

In a study on 5 workers currently exposed to TEL, no statistically significant correlation was found between PbB and atmospheric lead concentrations measured with personal samplers (Cope et al., 1979).

In workers moderately exposed to TEL and TEM the mean urinary levels of lead were 90 to 150 μg/l with a maximum of 310 μg/l (De Treville et al., 1962; Robinson, 1974a; Robinson, 1976).

Lead excreted with the urine consists essentially of organic lead, mainly diethyl lead. This finding results from a study made on 6 subjects with acute intoxication who were examined at varying intervals (from 3 to 20 days) after accidental exposure to TEL (Chiesura, 1970). Determination of lead in urine was made using the method proposed by Bolanowska (1968) to differentiate triethyl lead (benzene-extractable), Pb^{++} ion (precipitable as oxalate) and alkyl lead compounds in general (not precipitable as oxalate). Similar results were reported in a study by Yamamura et al. (1982) on a worker with acute TEL intoxication. The patient was a 48 years old man and urinary lead determinations were done serially between 20 and 196 days after the exposure and blood lead determination was performed once a week. Twenty days after the accident the blood lead level was 52.3 μg/100 ml and the urinary lead concentration was 586 μg/l.
In this case determination of the various chemical forms of lead (by atomic absorption after specific treatment and extraction) consisted of 50% diethyl lead, 48% inorganic lead and about 2% triethyl lead. On the 28th day, total lead consisted of 85% inorganic lead, 10% diethyl lead and 5% triethyl lead (Fig. 1).

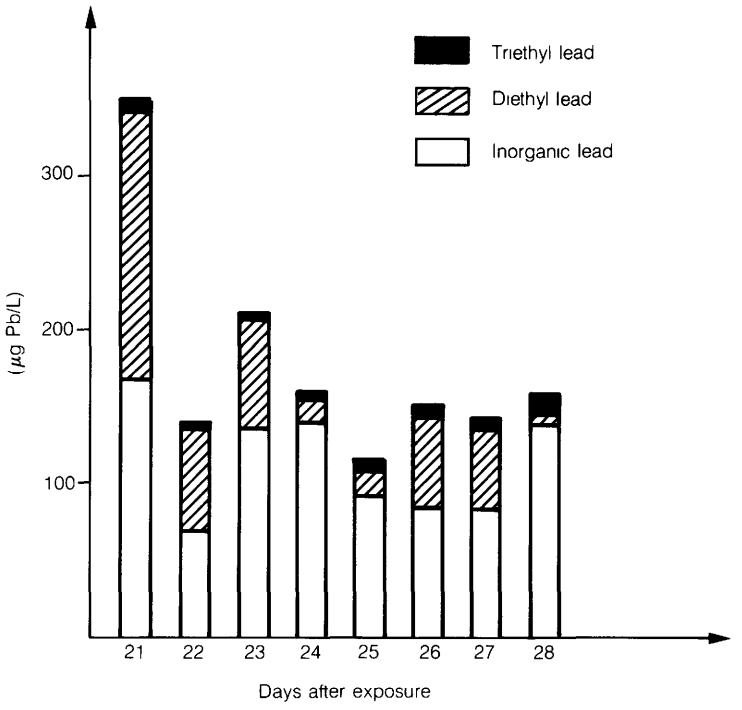


Fig. 1 Urinary concentrations of triethyl lead, diethyl lead and inorganic lead in a case of TEL poisoning.

It should be observed, however, that the patient had probably undergone chelation therapy on the 4th and 10th day as appears from the graph illustrating this paper.

A relationship seems to exist between urinary lead levels and appearance of symptoms of TEL intoxication. In 49 workers with no clinical signs of intoxication, lead in urine was, in the majority of cases, below 200 μg/l and only exceptionally exceeded 300 μg/l. In 45 subjects with evident symptoms of intoxication PbU was never lower than 200 μg/l and in many cases reached much higher levels, up to 850 μg/l (Foà et al., 1970).

Chiesura and Danieli (1969) observed that, when urinary lead exceeds 200 μg/l, there is a marked increase in the incidence of gastro-intestinal symptoms (loss of appetite, nausea, vomiting, gastric complaints, bad taste in the mounth) and neuropsychic symptoms

Table 1. TEL distillation workers: % frequency of signs and symptoms compared to PbU

PbU ug/l	N. cases	G.I. (1) symptoms %	Neuropsychic symptoms (2) %	CNS (3) alterations %	Arterial (4) hypotension %	Weigth loss (5) %	Pallor %	Diarrhoea and/or constipation %	No signs or symptoms %
<110	34	—	—	—	6	—	3	—	94.1
110-150	152	7.2	10	10	4.5	8.5	3.2	0.7	71.7
160-200	211	10.9	14	9	3.8	10	4.2	2.3	70
210-250	251	21.1	24.3	12.3	6.7	17	7.5	4.7	53
260-300	90	24.4	31	28	16.6	34.4	10	10	39
310-350	57	42.1	38.6	30	8.7	36.8	7	12.2	28
360-400	25	36	48	32	16	28	8	12	40
410-450	17	35	47	35	—	41	6	24	35 3
>450	15	40	33.3	10	—	27	20	13	40

(1) Lack of appetite, nausea and vomiting, gastric trouble, bad taste in the mouth
(2) Asthenia, headache, insomnia, troubled dreams, dazed state, dizziness.
(3) Tremour, Romberg test fluctuations, restlessness, excitability, moodiness.
(4) Systolic pressure reduced by 15-20 mm Hg compared to 7-15 days previously.
(5) 2-3 Kg weight loss over a relatively short period (about 1 month).

from Chiesura and Danieli (1969)

(weakness, headache, insomnia, troubled dreams, dazed state, dizziness) (Table 1). The objective signs of intoxication (CNS alterations, weight loss, arterial hypotension) become particularly frequent when PbU exceeds 600 μg/l, according to Gherardi and Vidoni (1965).

On the question of "limit values" for PbU the ACGIH (1963) and Fleming (1964) suggest that a PbU level above 110 μg/l should be considered as an indicator of exposure above "normal"; when a value of 150 μg/l is registered, the worker must be removed from the job. Kehoe (1983) on the other hand, considers that a PbU level of 150 μg/l is indicative of "harmfull degree of lead absorption"; if the PbU values reach 180 μg/l, even if the subject is asymptomatic, he must be moved away from the job involving exposure.

More recently Grandjean (1984) raised doubts as to whether a value of 150 μg/l can adequately protect against adverse effects.

As far as the relationship between PbU and atmospheric concentrations of alkyl lead compounds is concerned, Lynch (1975) found that "results from fixed station samplers throughout the manufacturing areas had not correlated well with routine medical examinations and urinary excretion levels for lead ... No correlation could be established between personal and fixed station monitor results; however, the mobile units gave significantly higher values. An approximately linear relationship between breathing zone lead levels and urinary excretion was found when the sum of the weekly average organic and inorganic lead TLV coefficients were related to the corresponding average urine excretion".
However, these findings are not of practical importance since the author used, to evaluate the environmental exposure, a coefficient obtained with an unspecified method.

Cope at al. (1979) did not find any correlation between atmospehric lead levels and PbU.

PbU measured after i.v. $CaNa_2EDTA$ treatment was altered even some time after cessation of exposure to alkyl lead compounds. However, it is not possible with this test to establish whether the subject was exposed in the past to alkyl lead compounds or to inorganic lead.

Administration of 1 g EDTA i.v. in 45 subjects with TEL intoxication and base line PbU between 40 and 300 μg/l caused a marked increase in urinary lead levels (PbU — EDTA = 1407 ± 867 μg/24 h) (Foà et al., 1970).

In a more recent study made on a subject with acute TEL intoxication after administration of 1 g EDTA i.v. a 5-fold increase in urinary inorganic lead levels was observed, whereas no significant increase in elimination of diethyl and triethyl lead was seen (Yamamura et al., 1982).

Administration of $CaNa_2EDTA$ in treatment of TEL intoxication may therefore serve to accelerate elimination of inorganic lead from the organism, but it apparently does not have any effect on the elimination of triethyl and diethyl lead.

In intoxication by alkyl lead compounds the changes in heme synthesis are less marked than might be expected on the basis of the internal dose tests, especially PbU, probably because these compounds are slowly degraded to inorganic lead.

Miller et al. (1972) found a reduction in erythrocyte ALAD levels in 27 subjects exposed to TEL who had no symptoms of intoxication and who had normal ALAU levels. In the exposed subjects the ALAD levels were about one-third of the levels in the control group. The mean PbB levels were 42.5 ± 10 μg/100 ml (Table II).

Table 2. ALAD and PbB levels in subjects exposed to TEL and TEM and in a control group.

	Exposed subjects n = 27	Control group n = 9	p*
ALAD nM PBG/hr/10^{10} red cells	220 ± 80	677 ± 230	< 0.001
PbB ug/100 ml	42.5 ± 10	15 ± 5	< 0.01

* Statistical comparison was using Student's test for independent samples — from Millar et al. (1972)

In a group of 123 asymptomatic subjects exposed to TEL and TEM, Robinson (1974a) demonstrated a significant correlation between ALAU and PbU ($r = 0.52$; $p < 0.001$) and a linear type increase in the urinary metabolite. However, for corresponding levels of PbU the ALAU levels in these subjects were definitely lower than in workers exposed to inorganic lead (Fig. 2).

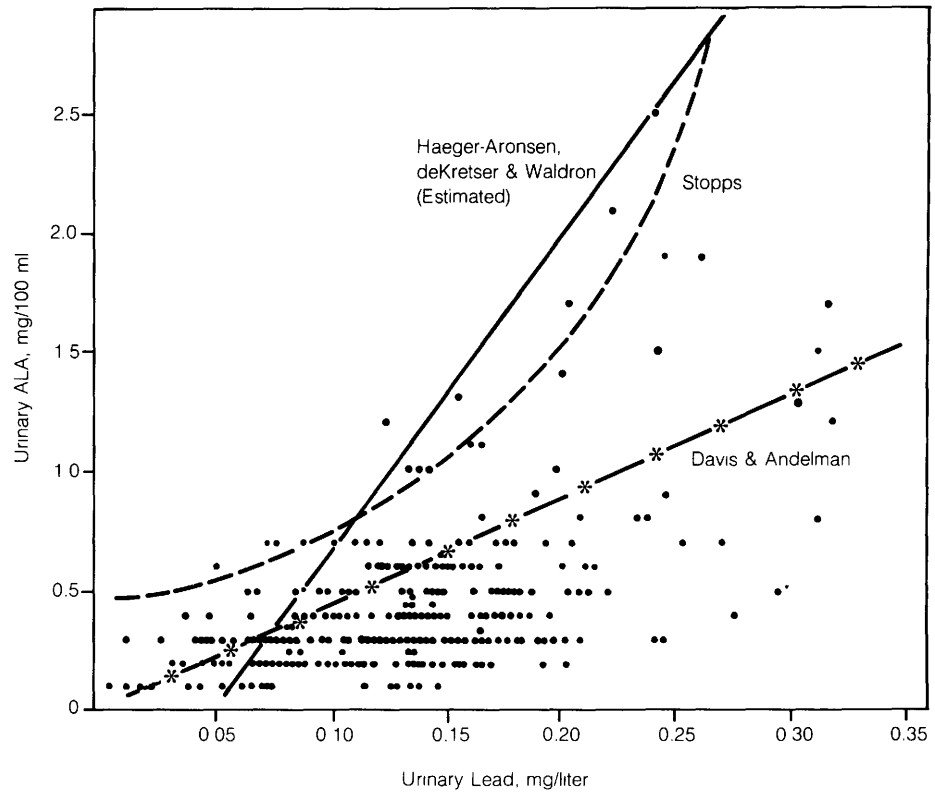


Fig. 2 Relationship between urinary lead and urinary ALA in organic lead workers (scatter diagram) and in workers exposed to inorganic lead (regression curves).

In subjects with signs and symptoms of moderately severe TEL intoxication Beattie et al. (1972) found normal ALAU levels, a slightly increase in EP and CPU and a clear reduction in ALAD. Contrary to the usual observations these subjects had high PbB levels which in one case exceeded 90 μg/100 ml. The baseline PbU levels were between 512 and 762 μg/l.

A study carried out on 13 subjects exposed to TEL for more than 8 years revealed normal EP levels ($\bar{x}$ = 21.12 μg/100 ml ± 2 s.d. 9.7). The PbB levels in the subjects who had clinical symptoms of intoxication such as headache and dizziness were between 62 and 155 μg/100 ml ($\bar{x}$ = 114.2 μg/100 ml) (Gutniak et al., 1964).

Foà et al. (1970) found abnormal EP levels in subjects who had ceased work involving exposure to alkyl lead compounds two months before. The EP levels and length of employment were significantly correlated and the highest EP levels were found in workers with the longest exposure to TEL.

Conclusions

It is clear from the above that PbU is the only biological test that can be used for the assessment of exposure in subjects working with organic lead compounds.
Various studies have in fact shown that a relationship exists between PbU levels and severity of symptoms. In addition, on the basis of studies reported in the literature, it seems that clinical symptoms appear when PbU exceeds 200 μg/l (Chiesura and Danieli, 1969).

It does not seem, however, that the test can be used to assess the degree of exposure through inhalation because the levels are not correlated (or only moderately correlated) with atmospheric lead concentrations; this could be due to the fact that the substance is effectively absorbed through the skin.

Need for Further Research

Relationship between PbU levels and state of health

Research should be undertaken on the performance of sensitive neurologic instrument tests and behavioural tests in subjects with occupational exposure of varying degrees, considering even low levels, to establish limit values.
Research of this type necessitates standardization of the methods of urine collection and standardization of the way the results are expressed.

Indicators of internal dose

Further research should be undertaken to ascertain:

- the existence of a possible correlation between lead content of total blood (PbB) and the portion of lead bound to the lipid fraction;
- the existence of a correlation between lead levels in the lipid fraction of the blood and symptoms;
- the existence of a possible correlation of the levels of organic lead in urine with PbB and with lead levels in the lipid fraction;
- the existence of a possible variation in the PbU levels during the course of the day, so as to establish the most appropriate time to collect urine samples;
- the behaviour of PbU after cessation of exposure if possible in conjunction with assessment of clinical symptoms;
- the relationship between the various chemical forms of lead present in urine (inorganic, triethyl and diethyl), so as to assess their behaviour both in currently exposed subjects and in subjects exposed in the past.

References

AHLBERG J., RAMEL C., WACHTMEISTER C.A. (1972) Organolead compounds shown to be genetically active. Ambio 1, 19
AKATSURA R. (1974) Tetramethyllead poisoning. Sangyo Igaky, 15, 3
BOLANOWSKA W. (1968) Distribution and excretion of triethyl lead in rats. Brit. J. Industr. Med. 25, 203
BOLANOWSKA W., PIOTROSKI J., GARCZYNSKI H. (1967) Triethylled in the biological material in cases of acute tetraethyllead poisoning. Arch. Toxicol. 22, 278
BOLANOWSKA W., WISNIEWSKA-KNYPL J.M. (1971) Dealkylation of tetraethyllead in the homogenates of rats and rabbits tissues. Biochem. Pharmacol. 20, 2108
BEATTIE A.D., MOORE M.R., GOLDBERG A. (1972) Tetraethylled poisoning. Lancet II, 12
CHIESURA P. (1970) Escrezione urinaria di cataboliti del piombo tetraetile nell'uomo. Med. Lavoro 61, 437
CHIESURA P., DANIELI A. (1969) Piomburia e giudizio di intossicazione da piombo tetraetile. Indagine in operai addetti alla produzione. Med. Lavoro 60, 129
COPE R.F., PANCAMO B.P., RINEHART W.E., TER HAAR G.L. (1979) Personnel monitoring of tetraalkyl lead in the workplace. Am. Ind. Hyg. Assoc. J. 40, 372
CREMER J.E. (1959) Biochemical studies on the toxicity of tetraethyllead and other organo-lead compounds. Brit. J. Industr. Med. 16, 191
CREMER J.E. (1984) Possible mechanism for the selective neurotoxicity in: Grandjean P.: Biological effects of organolead compounds. CRC Press Inc. Boca Raton, Florida (U.S.A.), p. 121
CREMER J.E., CALLAWAY S. (1961) Further studies on the toxicity of some tetra and trialkyl lead compounds. Brit. J. Industr. Med. 18, 277
DAVIS J.R., ANDELMAN S.L. (1967) Urinary delta-aminolevulinic acid (ALA) levels in lead poisoning. I. A modified method for the rapid determination of urinary delta-aminolevulinic acid using disposable ion exchange chromatography columns. Arch. Environ. Health 15, 53
DE KRETSER A.J., WALDRON H.A. (1963) Urinary delta aminolevulinic acid and porphobilinogen in lead exposed workers. Brit. J. Industr. Med. 20, 35
DE TREVEILLE R.T.P., WHEELER H.W., STERLING T. (1962) Occupational exposure to organic lead compounds. Arch. Environ. Health 5, 532
EUROPEAN ECONOMIC COMMUNITY DIRECTIVE, 78/611/EEC, July 22, 1978
FLEMING A.J. (1964) Industrial hygiene and medical control procedures. Arch. Environ. health 8, 266
FOA' V., CAVAGNA G., MANFREDI M. (1970) Valutazione della piomburia nella diagnosi di intossicazione da piombo tetraetile. Med. Lavoro 61, 491
GALZIGNA L., FERRARO M.V., MANANI G., VIOLA A. (1973) Biochemical basis for the toxic effects of triethyl lead. Brit. J. Industr. Med. 30, 129
GETHING J. (1975) Tetramethyl lead absorption: a report on human exposure to a high level of tetramethyl lead. Brit. J. Industr. Med. 32, 329
GHERARDI M., VIDONI G. (1969) Giorn. Med. leg. Infortunistica e tossicologia 11, 61 Quoted by Chiesura and Danieli (1969)
GRANDJEAN P., ANDERSEN O. (1982) Toxicity of lead additives. Lancet II, 333
GRANDJEAN P., NIELSEN T. (1979) Organolead compounds: environmental health aspects. Residue Reviews 72, 97
GRANDJEAN P. (1984) Organolead exposure and intoxications in: GRANDJEAN P.: Biological effects or organolead compounds, CRC Press Inc. Boca Raton, Florida (U.S.A.), p. 229
GUTNIAK O., KOSIOLOWA H., KOWALSKI E. (1964) Free protoporphyrin content of erythrocytes in chronic tetraethyl lead poisoning. Lancet I, 1137
HAEGER-ARONSEN B. (1960) Studies on urinary excretion of delta-amino-levulinic acid and other haem precursors in lead workers and lead intoxicated rabbits. Scand. J. Clin. Lab. Invest. 12 (suppl. 47), 1-128
HAMILTON A., REZNIKOFF P., BURNHAM G.M. (1925) Tetraethyl lead. JAMA 84, 1481
HEARD M.J., WELLS A.C., NEWTON D., CHAMBERLAIN A.C. (1979) Human uptake and metabolism of tetraethyl and tetramethyl lead vapour labelled with Pb-203. International Conference Management and Control of Heavy Metals in the Environment, London, 18-21 September 1979), p. 103
JENSEN A.A. (1984) Metabolism and toxicokinetics in: GRANDJEAN P.: Biological effects of organolead compounds. CRC Press Inc. Boca Raton, Florida (U.S.A.), p. 108
KEHOE R.A., THAMANN P. (1931) The behaviour of lead in the animal organism. II. Tetraethylled. Am. J. Hyg. 13, 478 (1931)
KEHOE R.A. (1964) Free protoporphyrin content of erythrocytes in chronic tetraethyllead poisoning. lancet II, 594
KEHOE R.A. (1983) Lead, alkyl compounds in: "Occupational Health and Safety", ILO, Geneva

KITZMILLER K.V., CHOLAK J., KEHOE R.A. (1954) Treatment of Inorganic Lead (TEL) Intoxication with CaEDTA. Arch. Industr. Hyg. 10, 312
LEAVITT F.H. (1934) Case oflead poisoning from gasoline impregnated with tetraethyl lead. Arch. Neutrol. Psychiatr. 32, 1109
LINCH A.L. (1975) Biological monitoring for industrial exposure to tetraalkyl lead. Am. Ind. Hyg. Ass. J. 34, 214
MACHLE W.F. (1935) Tetraethyllead intoxication and poisoning by related compounds of lead. JAMA 105, 578
MILLAR J.A., THOMPSON G.G, GOLDBERG A., BARRY P.S.I., LOWER E.H. (1972) Delta-aminolevulinic acid dehydrase activity in the blood of men working with lead alkyls. Brit. J. Industr. med. 29, 317
MIZOI Y., TATSUNO Y., HISHIDA S., MORGAKI T. (1973) Tetraalkyl lead poisoning. Japanese J. Leg. Med. 27, 371 (in Japanese)
MORTENSEN A. (1942) J. Ind. Hyg. 24, 285
Quoted by WALDRON and STOFEN in: Subclinical lead poisoning. Academic Pres, London-New York (1974)
MULLER K. (1953) Zur Kasuistik der Bleitetraethyl-Vergiftung. Zbl. Arbeit 3, 8
NIELSEN T., JENSEN K.A., GRANDJEAN P. (1978) Organic lead in normal human brains. Nature 274, 602
RAMEL C. (1973) The effect of metal compounds on chromosome segregation. Mutat. Res. 21, 45
ROBINSON T.R. (1974.a) Delta-aminolevulinic acid and lead in urine of lead antiknock workers. Arch. Environ. Health 28, 133
ROBINSON T.R. (1974.b) 20-year mortality of tetraethyl lead workers. J.O.M. 16, 601
ROBINSON T.R. (1976) The health of long service tetraethyl lead workers. J.O.M. 18, 31
SANDERS L.W. (1964) Tetraethyllead intoxication. Arch. Environ. health 8. 270
SKILLETER D.N. (1975) The decrease of mitochrondrial substrate uptake caused by trialkyltin and trialkylled compounds in chloride media and its relevance to inhibition of oxidative phosphorylation. Biochem. J. 146, 465
SPRINGMAN P., BINGHAM E., STEMMER K.I. (1963) The acute effects of lead alkyls. Arch. Environ. Health 6, 469
STOPPS G.J. (1968) Symposium on Air Quality Criteria: Lead. J. Occup. med. 10, 550
YAMAMURA Y., ARAI F., YAMAMUCHI H. (1982) Urinary excretion pattern of triethyllead, diethylled and inorganic lead in tetraethylled poisoning. Toxicovigilance industrielle. Actes du X Congrès du Medichem., Paris, 14-16 September 1982 (Part I, p. 57)

Biological indicators for the assessment of human exposure to industrial chemicals

Dimethylformamide

R. Lauwerys

Summary

DMF vapours are absorbed through the lung and also through the skin. Direct skin contact with DMF solution represents a frequent circumstance of exposure in industry.

DMF exerts its main toxic action on the liver and an early manifestation of excessive uptake is in the development of alcohol intolerance.

N-hydroxymethyl-N-methylformamide (DMF-OH) has been identified as a urinary metabolite of DMF. The concentration of N-methylformamide (NMF) in urine of workers exposed to DMF is much less than that of DMF-OH. The latter, however, is also measured as NMF by gas chromatography along with the small proportion of NMF present in urine.

Observations on workers have clearly demonstrated that for a substance like DMF, which can enter the organism not only by inhalation but also through skin contact, biological monitoring is much better than ambient monitoring for assessing exposure.

DMF-OH + NMF analysis in urine (both detected as a single NMF peak by gas chromatography) currently appears to be the best method to reach this goal. In view of their short biological half-life , it is recommended that the urine sample be collected at the end of the exposure period.

No meaningful biological threshold limit value can be proposed as yet but it has been shown that a concentration of DMF-OH + NMF not exceeding 40 to 50 mg/g creatinine in urine samples collected at the end of the workshift is not associated with signs of acute liver damage.

Dimethylformamide

Introduction

Chemical and Physical Properties

Dimethylformamide (DMF) is a colourless liquid at normal temperature. Several of its chemical and physical properties are given in Table I.

Table 1. Physico-chemical properties of dimethylformamide

*	Boiling point (760 mm Hg): 153°C
*	Vapour pressure (25°C): 3.7 mm Hg
*	Water solubility: infinite
*	Formula: $HC(=O)N(CH_3)_2$
*	1 ppm: 3 mg/m^3

Effects on Humans

The human data on the toxicity of dimethylformamide (DMF) are limited.
Prolonged skin contact may cause local irritation (Chary, 1974; Martelli, 1960; Potter, 1973; Reinl and Urban, 1965).
The main target organ following acute or long-term exposure to DMF is the liver (Potter, 1973; Reinl and Urban, 1965; Tolot et al., 1968); the gastric mucosa and the pancreas may also be affected (Chary, 1974). Nausea, vomiting, abdominal cramps, loss of appetite, hepatomegaly and increased activity of various serum enzymes (GOT, GPT, AP, OCT, γ-GT) have been reported in workers exposed to DMF for various periods of time.

In man, an early manifestation of excessive exposure is the development of alcohol intolerance (Chivers, 1978; Lyle et al., 1979). In workers exposed to DMF, various symptoms such as palpitations, anxiety, headache, flushing of the face and the trunk, nausea and even vomiting may occur when consumption of alcoholic beverages occurs during or within a few hours after the end of exposure.

The available data suggest that, provided skin contact is prevented, long term exposure to an airborne concentration below 10 ppm will not lead to the occurrence of biological signs of hepatic cytolysis (Krivanek et al., 1978; Lauwerys et al., 1980). Nevertheless, at this exposure level some individuals may still present symptoms of alcohol intolerance (Lauwerys et al., 1980; Yonemoto and Suzuki, 1980).

There is no published human data on the mutagenesis, teratogenesis or carcinogenesis of DMF.

Metabolism

Human data indicate that DMF absorption occurs not only through inhalation of vapours but also by direct skin contact with the liquid form (Maxfield et al., 1975; Kimmerle and Eben, 1975b; Lauwerys et al., 1980). We have found that in workers from an acrylic fibre factory skin absorption was more important than inhalation in the overall exposure to the solvent when no personal protective devices were used (Lauwerys et al., 1980).

DMF vapour can also be absorbed through contact with skin. Maxfield et al. (1975) have found that when a relatively inactive man exposes a large surface area to vapour concentrations around 10 ppm for 6 hours, cutaneous absorption may account for one quarter to one third of his total metabolite excretion during and for the 24 hours following the exposure.

DMF is rapidly metabolized in vivo. A negligible fraction of the absorbed dose is excreted unchanged in urine and in the gastrointestinal tract. Until recently, it was believed that the biotransformation of DMF in vivo, in rat, dog and human consisted of a progressive demethylation mediated by the microsomal mixed function oxidases to yield N-methylformamide (NMF) and formamide (F) (Kimmerle and Eben, 1975a,b; Krivanek et al., 1978; Lauwerys et al., 1980; Maxfield et al., 1975; Scailteur et al., 1981; Yonemoto and Suzuki, 1980).
It has now been demonstrated that the metabolite identified as NMF by gas chromatography (Barnes and Henry, 1974) is mainly N-hydroxymethyl-N-methylformamide (DMF-OH), a stable carbinolamine which breaks down in the injector of the gas chromatograph to give NMF (Scailteur et al., 1974; Scailteur and Lauwerys, 1984a,b). By analogy, N-hydroxymethylformamide (NMF-OH) is considered to be the metabolite initially described as F. Only a very small percentage of the absorbed DMF, however, is transformed into NMF and F (probably less than 5%).

The metabolic pathway leading from DMF to DMF-OH involves hydroxyl radicals. The slight amount of NMF produced in vivo does not seem to result from further DMF-OH biotransformation but comes directly from DMF (Scailteur and Lauwerys, 1984a,b). NMF is more toxic than DMF and the differences between DMF and NMF toxicity were difficult to explain when NMF was thought to represent the principal in vivo metabolite of DMF (Kimmerle and Eben, 1975a,b). The metabolic studies (Scailteur et al., 1984; Scailteur and Lauwerys, 1984a,b) which demonstrate that following DMF administration, the main urinary metabolite is in fact DMF-OH and not NMF, now offer a logical explanation for these apparent discrepancies, since DMF-OH has been shown to be less acutely toxic than NMF (Scailteur and Lauwerys, 1984b).

Kimmerle and Eben (1975b) exposed 4 men to 26 ± 8 ppm DMF for 4 hrs and 3 men and 1 woman to 87 ± 25 ppm DMF for 4 hrs.
Concentrations of DMF and its metabolites (mainly DMF-OH and NMF-OH measured as NMF and F respectively) in blood and urine were determined.
DMF was no longer detectable in the blood a few hours after exposure. Only after exposure to 87 ppm was it detectable in the urine.
DMF-OH (measured as NMF) was detectable in the urine 4 hours after beginning the exposure. The majority of the substance was eliminated within 24 hours.
The elimination of NMF-OH (measured as F) was delayed; it was detectable in urine up to 72 hours after beginning the exposure.

The same authors also exposed 4 men to 21 ± 4 ppm DMF for 4 hrs a day for 5 consecutive days. DMF concentration in the blood decreased rapidly and generally was no longer detectable 4 hours after the end of exposure. DMF-OH concentration (measured as NMF) in blood during repeated exposure varied from one person to another but accumulation did not occur. Urine analysis also showed that during repeated exposure to approximately 20 ppm DMF no accumulation of DMF-OH (measured as NMF) occurs in the body. During exposure, DMF-OH concentration in a 24 hours urine sample remains constant (± 30 mg/24 hr) but it was no longer detectable 48 hours after the last exposure.

The authors propose to determine DMF-OH (measured as NMF) concentration in 24 hr urine as a routine monitoring method for employees exposed to DMF. They conclude that excretion above 50 mg/24 hr urine indicates exposures exceeding 20 ppm.

Maxfield et al. (1975) have also exposed volunteers to DMF (vapours or direct skin application) and have measured the rate of urinary excretion of DMF-OH (measured as NMF). The vapour concentration was ± 10 ppm and exposure lasted for 6 hours. The 4 volunteers were dressed in shorts, socks and shoes to provide nearly maximal surface area for cutaneous absorption. Only skin absorption occurred when a mask was worn but

absorption through both the lungs and skin occurred when no mask was worn. In the case of direct skin application, the subjects wore a mask to avoid inhaling vapour and the amount of undiluted DMF applied to the skin was 0.3 ml.

The type of exposure appeared to determine how soon DMF-OH (measured as NMF) appeared in measurable amounts in the urine. It appeared most promptly when DMF vapour was absorbed through both lungs and skin and almost as quickly when DMF liquid was applied to the skin, but later when DMF vapour was absorbed only through the skin. In the first exposure condition, the peak excretion rate occurred within 5 hours after the beginning of exposure, whereas in the latter condition, it was not reached before 15 hours. Usually, DMF-OH (measured as NMF) had disappeared from the urine on the morning after the skin exposure to liquid DMF and from samples collected 24 to 26 hours after the start of the exposure to DMF vapour, with and without a mask. For exposure to DMF vapour at the TLV concentration (30 mg/m^3 or 10 ppm), absorption through the lungs accounted for most (61 to 86%) of the total metabolite excreted during the 24 hour interval following the exposure. Exposure of large areas of skin to this concentration of DMF vapour can, however, result in the absorption of significant amounts of the compound through the skin.

Only a small amount of DMF inhaled or in contact with the skin may be recovered in the urine as DMF-OH (measured as NMF). In their experiments, the estimated dose recovered ranged from 0.5 to 2%. The authors conclude that the amount of metabolite in isolated urine specimens when collected at a specified time in relation to the work day may serve to monitor overexposure to DMF.

According to their data, the total amount of DMF-OH (measured as NMF) excreted in the urine following 6 hours exposure to 10 ppm DMF is lower than 5 mg. Similar results were obtained by Krivanek et al. (1978). They exposed 8 subjects to DMF vapour at an average concentration of 8.8 ppm for 6 hrs daily for 5 consecutive days. The amount of DMF-OH (measured as NMF) excreted during each 24 hr interval following the beginning of exposure amounted to about 2.5 mg.

In summary, the volunteer studies indicate that DMF-OH (measured as NMF) appears rapidly in the urine of humans exposed to DMF; its biological half-time is short, probably around 12 hrs. Unfortunately, the quantitative data obtained by Kimmerle and Eben (1975b) and Maxfield et al. (Maxfield et al., 1975; Krivanek et al., 1978) are not in good agreement since the values reported by the latter researchers appear to be about 5 times lower than those expected on the basis of the results obtained by Kimmerle and Eben (1975b).

Factors affecting DMF metabolism

Eben and Kimmerle (1976) studied the metabolic interaction between DMF and ethanol in rats, dogs and in men.

A delayed biotransformation of DMF was observed in rats and dogs when ethanol (2.0 g/kg) was administered orally prior to acute exposure to DMF vapours (rats: 209, 104 and 87 ppm for 2 hrs; dogs: 210-240 ppm for 2 hrs). Thus, the DMF concentration in the blood was 2 to 6 fold higher in the animals pretreated with ethanol than in control animals. The administration to rats of a lower oral dose of ethanol (0.2 g/kg) did not influence the metabolism of DMF.

During repeated exposure to DMF (200 ppm 2 h/day on 5 consecutive days) and ethanol (2.0 g/k p.o. once a day for 5 consecutive days) the biotransformation of DMF was also inhibited. Furthermore, they found that in animals, the oxidation of ethanol was also influenced by DMF.

The authors attempted to confirm the interaction on volunteers. Four persons were exposed to 50-80 ppm DMF for 2 hours alone or with previous administration of ethanol (19 g/person). A comparatively lower DMF-OH concentration (measured as NMF) in blood after ethanol administration indicates that ethanol can also influence the metabolism of DMF in man. The comparison of the ethanol and acetaldehyde concentration in the blood after ethanol administration with or without exposure to DMF (82 ± 20 ppm for 2 hrs) did not produce definite results since in both conditions acetaldehyde was not detected in the blood.

We have, however, indicated above that an antabuse-like effect of DMF is a frequent observation in workers exposed to this solvent. This may be due to an inhibition of alcohol deshydrogenase by DMF (Sharkawi, 1979).

Biological Indicators

Several biological parameters can be considered for the evaluation of exposure to dimethylformamide.

- DMF in blood
- DMF in expired air
- The sum of N-hydroxymethyl - N-methylformamide (DMF-OH) and N-methylformamide (NFM) in blood. Both metabolites are measured as NMF by gas chromatography.
- The sum of DMF-OH and NMF in urine. Both metabolites are measured as NMF.
- The sum of N-hydroxymethylformamide (NMF-OH) and formamide (F) in urine. Both metabolites are measured as F.

DMF in blood

DMF can be measured in blood (Kimmerle and Eben, 1975a). From data published by Kimmerle and Eben (1975b) it appears that DMF concentration in blood increases continuously during exposure and disappears rapidly after the end of exposure.
At the end of a 4-hour exposure to average atmospheric concentrations of 21 and 87 ppm, the mean DMF concentration in blood amounted to about 0.3 and 1.4 mg/100 ml respectively.
The time of sampling is very critical, which limited the usefulness of the test for the routine control of workers.

DMF in expired air

When skin contact with DMF can be prevented, there is during exposure a significant correlation between environmental exposure and DMF concentration in alveolar air. According to Brugnone et al. (1980), the alveolar concentration amounts on average to 3 mg/m^3 when the atmospheric concentration of DMF is 10 mg/m^3.

Sum of DMF-OH and NMF in blood

As indicated above, DMF-OH represents the main oxidative metabolite of DMF, NMF being a minor metabolite. The gas chromatographic method initially developed for NMF determination, measures both NMF and DMF-OH which is demethylated to NMF in the injector of the gas chromatograph.

Unlike DMF, the level of DMF-OH in blood (measured as NMF) seems to remain fairly constant for a few hours after the end of exposure (Kimmerle and Eben, 1975b).
The only data available relating DMF-OH (+ NMF) concentration in blood and exposure to DMF are those of Kimmerle and Eben (1975b) based on 4 volunteers exposed to DMF vapours at concentrations ranging from 21 to 87 ppm. At the end of the exposure, DMF-OH (+ NMF) in blood amounted on the average to 0.2 mg/100 ml (21 ppm) and 0.6 mg/l (87 ppm).
More data on humans are required to evaluate the relationship of this parameter with the intensity of exposure.

Sum of DMF-OH and NMF in urine

In workers exposed to DMF, DMF-OH constitutes the main urinary metabolite; only a trace amount of NMF is present in urine. However, the gas chromatographic technique used for urine analysis cannot distinguish between both metabolites since DMF-OH is demethylated to NMF at high temperature (see above).
Data available from both human volunteers and workers exposed have demonstrated that DMF-OH (+ NMF) analysis in urine is useful for monitoring exposure to DMF. However, the results permitting to relate exposure to DMF, and amount of metabolites (DMF-OH + NMF) excreted are still very limited.
Furthermore, the results of Maxfield et al. (1975) indicate that individual variation in metabolite excretion is high, probably resulting from differences in the actual amount absorbed as well as factors such as the rate of metabolism and/or renal clearance.
We have already indicated that according to Maxfield et al. (1975) data based only on 4 volunteers, the total amount of DMF-OH + NMF excreted in the urine following a 6-hour exposure to 10 ppm DMF vapour is on the average less than 5 mg (range 2 to 5.75 mg).

The results obtained by Kimmerle and Eben (1975b) on 4 volunteers are summarized in Table II.

Table 2. Urinary excretion of DMF-OH plus NMF in 4 volunteers exposed to DMF vapour.

Exposure	mg DMF-OH + NMF excreted within 24 hours after beginning of exposure			
	Subject 1	Subject 2	Subject 3	Subject 4
26 ppm 4 hr	26	21	27	22
87 ppm 4 hr	94	90	111	95
21 ppm 4 hr (5 consecutive days)	on the average 25 to 30 mg per each 24 hr period (4 subjects)			

Catenacci et al. (1980) Yonemoto and Suzuki (1980) and Wicarowa and Dadah (1980) examined workers exposed to dimethylformamide and found a relationship between the environmental concentration of the solvent and the amount of DMF-OH + NMF (both measured as NMF) excreted in 24 hours. Their quantitative results, however, are very different since, for an exposure to 10 ppm DMF, the first group of authors found an excretion of 12 - 15 mg DMF-OH + NMF per 24 hours, whereas Yonemoto and Suzuki (1980) and Wicarova and Dadah (1981) reported an average excretion of 5 and 6 mg of DMF-OH + NMF per 24 hours, respectively, for the same exposure level.
Differences in the percutaneous absorption of DMF and in alcohol consumption, which interferes with DMF metabolism, might explain this discrepancy.

Lauwerys et al. (1980) have carried out a field study in an acrylic fibre factory. DMF vapour concentration was measured at different workplaces with static samplers during a week and the time spent by each worker at different places during each working day was estimated. For each worker and for each day an integrated exposure (concentration x duration) was calculated. It should, however, be recognized that during some operations, skin contact also occurred and that the airborne concentration did not necessarily reflect the total exposure. Urine samples were collected immediately before and after the shift for 5 or 6 consecutive days.
Several observations previously made on volunteers were confirmed:

1. The sum of DMF-OH + NMF (both measured as NMF) in urine is a sensitive biological parameter of exposure. The presence of metabolites (mainly DMF-OH) in urine can be easily detected even when the average airborne DMF concentration is below 30 mg/m^3, the current ACGIH TLV (integrated exposure: 180 $mg.h.m^{-3}$).
2. The metabolism of absorbed DMF is rather rapid: DMF-OH + NMF concentration in urine is usually greater at the end of the shift than in the morning of the next day.
3. In a group of workers the sum of DMF-OH + NMF in urine at the end of the shift seems to reflect the intensity of exposure of the same day.

On an individual basis ($N = 116$) there was a very low correlation between integrated exposure and DMF-OH + NMF concentration (expressed in mg/g creatinine) in urine collected at the end of the shift ($r = 0.24$ $P < 0.010$) or before ($r = 0.16$ $P > 0.05$) resuming work the next day (i.e. ± 16 hours after the end of the work).

This could be due to individual variation in metabolism (mainly rate of excretion) but also to an error in the estimation of the total exposure since we know that in this type of industry skin contact with DMF solution is an important route of absorption. This was clearly demonstrated by comparing the urinary excretion of DMF-OH + NMF (both measured as NMF by gas chromatography) in workers using different protective devices (Fig. 1). The metabolite concentration in urine collected at the end of a day when the workers were equipped with a self-contained breathing apparatus but did not protect their skin (i.e. first day of week 3, in Fig. 1) was about three times higher than during the period when only their skin was protected with gloves (Fig. 1).

The results of the health screening of these workers also suggested that a concentration of DMF-OH + NMF (both measured as NMF by gas chromatography) in urine samples collected at the end of the workshift not exceeding 40 to 50 mg/g creatinine is not associated with signs of acute liver damage (Lauwerys et al., 1980). Such exposure, however, may still be associated with signs of alcohol intolerance. These results also demonstrate very clearly that for a substance like DMF, which can enter the organism not only by inhalation but also through skin contact, biological monitoring is much better than ambient monitoring in assessing exposure.

Dixon et al. (1983) have noted a seasonal variation in the urinary concentration of DMF-OH + NMF (both measured as NMF) in workers exposed to DMF. This change was attributed to seasonal differences in urine volume. Twenty-four hour urine volumes were on the average 13% lower in hot weather than in cold weather.

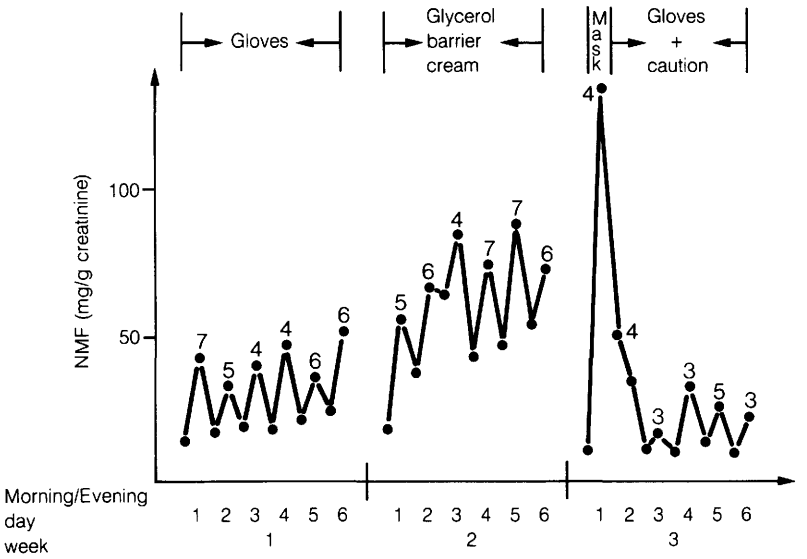


Fig. 1 Urinary excretion of DMF – OH + NMF (both measured as NMF by gas chromatography) in workers using different protective devices.

Sum of NMF-OH and F in urine

The experiments on volunteers exposed to DMF vapours (Kimmerle and Eben, 1975b) suggest that the excretion of NMF-OH + F (measured as F) in urine is dose-dependent. However, the excretion of NMF-OH + F is slightly delayed. The highest concentration was found between 4 and 20 hours after the end of the test. As indicated above, however, the concentration of NMF-OH + F is also lower than that of DMF-OH + NMF.

Conclusion

Since in industrial settings the skin represents an important, if not the major route of DMF entry into the organism, the possibility of assessing exposure to this solvent by a biological method is obvious. It represents the only approach to estimating the total uptake.
Among the biological tests which have been considered for evaluating the intensity of exposure to DMF, the determination of DMF-OH + NMF (both measured as NMF by gas chromatography) in urine appears to be the most practical.

References

BARNES J.R. and HENRY N.W. III (1974) The determination of N-methylformamide and N-methylacetamide in urine. *Am. Ind. Hyg. Assoc.* (February) J. 84-87

BRUGNONE F., PERBELLINI L., GAFFURI E. (1980) N-N-dimethylformamide concentration in environmental and alveolar air in an artificial leather factory. *Brit. J. Ind. Med.* **37,** 185-188

CATENACCI G., GHITTORI S., COTTICA D. et al. (1980) Occupational exposure to dimethylformamide and urinary excretion of monomethylformamide. *G. Ital. Med. Lav.* **2**, 53-60 (in Italian)

CHARY S. (1974) Dimethylformamide, a cause of acute pancreatitis ? *Lancet* **2**, 356

CHIVERS C.P. (1978) Disulfiram effect from inhalation of dimethylformamide. *Lancet* **1**, 331

DIXON S.W., GRAEPEL G.J., LOONEY W.C. (1983) Seasonal effects on concentration of monomethylformamide in urine samples. *Am. Ind. Hyg. Assoc. J.* **44**, 273-275

EBEN A., KIMMERLE G. (1976) Metabolism studies on N,N-dimethylformamide. III. Studies about the influence of ethanol in persons and laboratory animals. *Int. Arch. Occup. Environ. Health* **36**, 243-266

KIMMERLE G. and EBEN A. (1975a) Metabolism studies of N,N-dimethylformamide. I. Studies in rats and dogs. *Int. Arch. Arbeitsmed.* **34**, 109-126

KIMMERLE G. and EBEN A. (1975b) Metabolism studies of N,N-dimethylformamide. II. Studies in persons. *Int. Arch. Arbeitsmed.* **34**, 127-136

KRIVANEK N.D., McLAUGHLIN M., FAYERWEATHER W.E. (1978) Monomethylformamide levels in human urine after repetitive exposure to dimethylformamide vapor. *J. Occup. Med.* **20**, 179-182

LAUWERYS R., KIVITS A., LHOIR M., RIGOLET P., HOUBEAU D., BUCHET J.P., ROELS H.A. (1980) Biological surveillance of workers exposed to dimethylformamide and the influence of skin protection on its percutaneous absorption. *Int. Arch. Occup. Environ. Health* **45**, 189-203

LYLE W.H., SPENCE T.W.M., McKINNELEY W.M., DUCKERS K. (1979) Dimethylformamide and alcohol intolerance. *Brit. J. Ind. Med.* **36**, 63-66

MARTELLI D. (1960) Tossicologia della dimetilformamide. *Med. Lavoro* **51**, 123-127

MAXFIELD M.E., BARNES J.K., AZAR A., TROCHIMOWICZ H.T. (1975) Urinary excretion of metabolite following experimental human exposure to DMF and DMAC. *J. Occup. Med.* **17**, 506-511

POTTER H. (1973) Dimethylformamide induced abdominal pain and liver injury. *Arch. Environ. Health* **27**, 340-341

REINL W., URBAN H.J. (1965) Erkrankungen durch Dimethylformamid. *Archiv Gewerbepath. Gewerbehyg.* **21**, 333-346

SCAILTEUR V., BUCHET J.P., LAUWERYS R. (1981) The relationship between dimethylformamide metabolism and toxicity. In: Brown S.S., Davies D.S. (eds.) "Organ directed toxicity, chemicals indices and mechanisms", Pergamon Press, Oxford, 169-174

SCAILTEUR V., de HOFFMANN E., BUCHET J.P., LAUWERYS R. (1984) Study on in vivo and in vitro metabolism of dimethylformamide in male and female rats. *Toxicology* **29**, 221-234

SCAILTEUR V., LAUWERYS R. (1984a) In vivo and in vitro oxidative biotransformation of dimethylformamide in rat. *Chem. Biol. Interactions* **50**, 327-327

SCAILTEUR V., LAUWERYS R. (1984b) In vivo metabolism of dimethylformamide and relationship to toxicity in the male rat. *Arch. Toxicol.* **56**, 87-91

SHARKAWI M. (1979) Inhibition of alcohol dehydrogenase by methylformamide and dimethylsulfoxide. *Tox. Lett.* **4**, 493-497

TOLOT F., ARCADIO F., LENGLET J.P., ROCHE L. (1968) Intoxication par la diméthylformamide. *Arch. Mal. Prof.* **29**, 714-717

WICAROVA O., DADAH O. (1981) N-methylformamide levels in urine of persons exposed to dimethylformamide vapours. *Prac. Lek.* **33**, 42 (in Czeck)

YONEMOTO J., SUZUKI S. (1980) Relation of exposure to dimethylformamide vapour and the metabolite, methylformamide, in urine of workers. *Int. Arch. Occup. Environ. Health* **46**, 156-165

Biological indicators for the assessment of human exposure to industrial chemicals

Mercury

V. Foà, G. Bertelli

Summary

The characteristics of the metabolism and toxicology of mercury vapours and inorganic compounds are substantially different compared with organic compounds. Metallic mercury is widely used in industry. In chronic exposure the main alterations involve the central nervous system. The main route of absorption in occupational exposure is via the respiratory apparatus. Mercury easily passes the blood-brain barrier and accumulates in the brain. Mercury is mainly eliminated with the urine. Determination of mercury in urine is at present the most widely used indicator of exposure; values calculated on a group basis were found to be well correlated with the environmental concentrations. However, the correlations can be established only after about one year from the beginning of exposure, when the binding sites in the kidney are saturated. It should be borne in mind that although HgU is an indicator of exposure, it does not permit evaluation of the dose in the critical organ (central nervous system). Fewer studies have been made on the behaviour of HgB. It has been found that the levels of this indicator are correlated with the levels of HgU in occupationally exposed subjects. At present no biological tests are available that can be used for evaluation of early reversible effects due to mercury.

Due to the high toxicity of organic mercury compounds, especially alkyl mercurials, their use in industry has been banned in many countries. Non-occupational exposure to these compounds, via ingestion of contaminated food, does, however, occur to a considerable extent. The digestive apparatus is the most effective route of absorption and elimination occurs via the faeces. Organic mercury compounds easily cross the blood-brain barrier causing serious alterations to the central nervous system. The measurement of HgB is a useful test for evaluating the degree of exposure, but measurement of HgU is of no practical use. No indicators of early biological effect exist for alkyl mercury compounds.

Mercury

Introduction

Physico-chemical Properties

Mercury exists in nature in liquid state at ambient temperature. Atomic weight : 200.6, atomic number : 81, density : 13.6, melting point : -38.9°C. Oxidation state is +1 and +2.
Mercury vapours are considered to be insoluble in water; solubility in liquids at ordinary temperatures is in the order of 5-50 mg/l.
Mercury salts such as alides, sulphates and nitrates are soluble in water. Mercury is contained in the earth's crust mainly in the form of a sulphide; the red sulphide or cinnabar is the ore richest in mercury, containing up to 70% (Berlin, 1979).
Mercury is capable of forming organic compounds such as alkyl-, phenyl- and methoxylalkyl mercurials. However, the distinction between organic and inorganic compounds has more of a didactic or chemical value than a toxicological basis. Within these two groups, for example, the toxic properties of elemental mercury vapours differ from those of inorganic mercury salts; moreover, the adverse effects of the alkyl mercurials have been shown to differ dramatically from the other organic mercury compounds.

The industrial consumption of mercury, which amounted to less than 8000 tons/year in 1980, appears to be slowly but constantly declining. Consumption of mercury in the European Common Market was 1164 tons in 1982. Distribution in the various industrial sectors in the period 1977-78 was : over 60% in the chemical industry (chlor-alkalis, paints), 12% in the accumulator industry, 7% in the precision instruments industry, and the remaining 20% in miscellaneous uses (Fig. 1) (Eurostat, 1979-82)

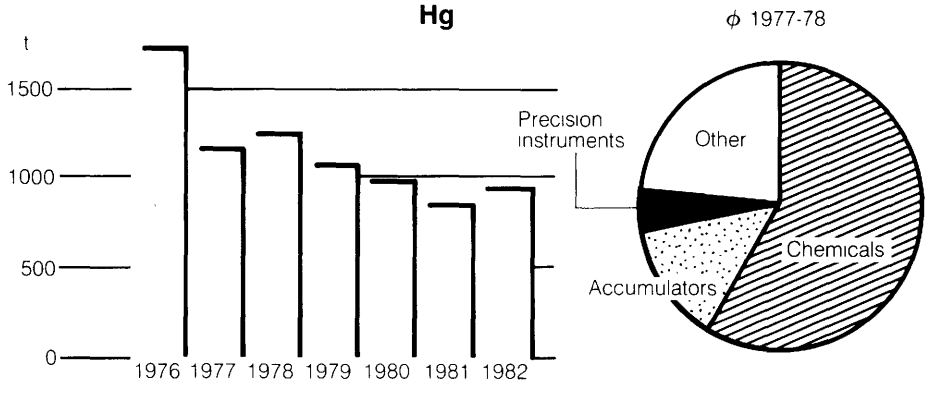


Fig. 1 Consumption of mercury in EEC countries and distribution in various industrial sectors (From Eurostat, 1979-82)

Organic mercury compounds such as the methylmercurials and ethylmercurials are widely used in agriculture as fungicides; however, the majority of the industrialized countries have banned their use (Berlin, 1979).

Effects on Humans

Acute occupational intoxication by metallic mercury is rather rare and occurs following inhalation of large quantities of the vapours. The effects are seen on the respiratory apparatus, varying from slight bronchial irritation to pulmonary oedema leading to fibrosis (Browning, 1969; Hamilton and Hardy, 1974; Seaton and Bishop, 1978; Berlin, 1979). Rare cases of "mercurial pneumonia" have been described even following exposure to environmental mercury concentrations between 1 and 3 mg/m^3 (Friberg and Nordberg, 1972).

In chronic occupational intoxication the target organ is the central nervous system. The pronounced clinical symptoms are characterized mainly by tremour and psychic erethismus. Static and intentional tremour, with serious emotional aggravation is initially slight and hardly noticeable, involving only the face; it becomes progressively more evident, involving the limbs, and can hinder normal activities such as eating, dressing and walking. There are evident changes in handwriting and speech monotonous, syllabized, stammering (Hamilton and Hardy, 1974; Baldi, Vigliani and Zurlo, 1953; Foà, 1977; Berlin, 1979). Psychic erethismus has been described as irritability, changes in sociableness, anxiety, insecurity, loss of memory (Hamilton and Hardy, 1974; Berlin, 1979). Sometimes these symptoms are the only signs of early mercury poisoning so that some authors have used the term micromercurialism (Berlin, 1979).

Cases of polyneuritis have been described in workers with mercury intoxication. In addition to significant electroneurographic evidence of motor conduction velocity impairment in workers, clinical findings indicating alterations of both sensory and motor nerve fibres have been reported. These findings have been explained as the result of the toxic effect of mercury on the anterior horn motor neurons with axonal degeneration (Vroom and Greer, 1972; Gilioli et al., 1976; Levine et al., 1982).
The central nervous system is seriously involved particularly in intoxication by organic compounds, giving rise to sensory and motor nerve effects (leading even to generalized ataxy), sight defects with concentrical restriction of the visual field and, in the most serious cases, blindness, deafness, drowsiness and coma (Al-Damluji, 1976; Skerfving and Vostal, 1972). Involvement of the peripheral nervous system has also been described (Skerfving and Copplestone, 1976). In ethylmercury poisoning the damaging effects on the kidney are of a peculiar and specific nature (Al-Damluji, 1976).
Serious outbreaks of collective methylmercury intoxication occurred in Minamata and Niigata in Japan (700 cases) and Iraq (1600 cases) following ingestion of contaminated fish and cereals.

Mutagenesis

An increased frequency of aneuploid cells was observed in the lymphocytes of workers exposed to mercury vapours (Verschaeve et al., 1976). The same authors also observed chromosomal aberrations in workers exposed to ethylmercury. Skerfving et al. (1974) found chromosomal aberrations in the lymphocytes of subjects who had eaten fish contaminated with methylmercury proportionate in number to the dose ingested.

Other authors did not find an increased incidence of chromosomal aberrations in subjects suffering from accidental methylmercury poisoning, compared to a control population (Baghdad Meeting, 1974).

An increased incidence of chromosomal aberrations not accompanied by any significant variations in frequency of aneuploid or polyploid cells or sister chromatid exchanges was found both in workers exposed to metallic mercury and in workers exposed to a mixture of methylmercury and ehtylmercury (Popescu et al., 1979).

Metabolism

Absorption, distribution and excretion of mercury vary considerably according to the chemical form it takes.

Absorption

Metallic mercury (Hg°) is the form most widely encountered in occupational exposure. Mercury vapours and dusts or aerosols of inorganic mercury are absorbed via the respiratory tract in amounts or at sites depending upon their particle size and solubility in biological fluids.

Absorption by the inhalation route is a highly efficient process. In fact, the affinity of mercury for proteins and all other substances with a sulphydryl group explains the capacity of the

metal for immediate reaction with the active groups of the respiratory mucosa, which has a surplus of sulphydryl groups, compared with the relatively small quantities of mercury inhaled. Equilibrium between Hg in air and in plasma is reached within a very short time from the beginning of exposure, and 20 hours after cessation of exposure the lung contains practically no more of the metal.
The percentage of lung retention following a single exposure (subjects exposed to concentrations of 100 $\mu g/m^3$ of labelled mercury) varies from 74 to 76% when inspiration is through the nose, to 50% when inspiration and expiration is through the mouth; the retention percentage remains constant in time even if exposure continues (Hursh et al., 1976; Teisinger and Fiserova Bergorova, 1965).

In occupational exposure, absorption by ingestion can be considered as accidental. Nevertheless, if the vapours inhaled are at a higher temperature than the body temperature, they may condense on the mucosa of the mouth and upper respiratory tract and then be swallowed. If elemental mercury is ingested, however, the oxidation process in the intestinal tract is usually too slow to be completed before the mercury is eliminated with the faeces (Clarkson, 1972, I and II). Inorganic mercury and its salts are absorbed very slightly by the intestinal tract; the portion absorbed is calculated as being 2% to 10% (Clarkson, 1971; Rahola et al., 1973).

If conveyed by organic solvents, the inorganic compounds could be absorbed via the undamaged skin, where part of the mercury could be converted to elemental mercury and there deposited.
Organic compounds, however, are easily absorbed via the intestines: between 50% and 80% of the ingested doses, with peaks up to 94-95% in the case of methylmercury, is absorbed by this route.

Distribution

The distribution of mercury in the body tends to establish a state of equilibrium determined by the following factors : concentration of mercury compounds in the various blood compartments; related concentrations of free sulphydryl groups; affinity of the cellular components for mercury; velocity of association and dissociation of the mercury-protein complex. Ninety-five percent of the sulphydryl group of the blood are found in the red blood cells: 90% in the globin, 4% in the membrane, and the remainder in the reduced glutathione; the albumin in the plasma has the largest supply of sulphydryl groups.
If the mercury compounds were equally distributed among the sulphydryl groups of the blood, the highest portion should be bound to the red blood cells. However, considerable differences are recorded among the different mercury compounds and among different animal species, just as distribution also varies among the different blood components according to the dose and length of exposure.

Generally speaking, however, it can be said that, while **90% of the organic compounds are transported inside the red blood cells, about 50% or more of inorganic mercury is transported into the plasma, especially bound to the albumin.**
Also, the distribution of mercury in the various compartments of the body depends on the dose, length of exposure, degree of oxidation of mercury, and the type of compound to which mercury is bound (Brown and Kulkarni, 1967).

When mercury vapours are inhaled, oxidation of elemental mercury is completed partly in the blood, mostly in the erythrocytes (Clarkson, 1979) and partly in the tissues by means of a catalase (Nielsen-Kudsk, 1971; Magos, 1974). However, alongside the portion of mercury present in oxidised form, there is a portion in the blood in a not yet ionized state (Magos, 1967, 1968). The diffusion of the latter portion from the blood into the tissues and through the cell membranes is facilitated by its greater liposolubility and by the lack of any electrical charge, allowing it to pass the blood-brain barrier.

Consequently, the levels of mercury in the brain are 10 times higher following exposure to vapours of elemental mercury compared with administration of identical quantities of ionized mercury in various animal species (Berlin et al., 1966; Berlin et al., 1969).

After absorption, elemental mercury, oxidized into mercury ion, behaves toxicologically like that ion (Friberg and Nordberg, 1972; Friberg and Vostal, 1972).

A study of the distribution of elemental mercury in the central nervous system in rats and mice (Cassano et al., 1966) revealed a greater mercury concentration in the grey matter than in the white, with the highest levels in certain neurones of the cerebellum, of the spinal cord, medulla, pons and mid-brain. In the cerebellum, a selective localization was noted in the Purkinje cells and in the neurones of the dentate nucleus.
Available data from long-term exposure of squirrel monkeys (Berlin, 1979) and a few clinical cases of occupational exposure (Takahata et al., 1970; Watanabe, 1971) indicate that

mercury is accumulated in the cerebral cortex, especially in the occipital and parietal cortical areas.
In other organs, the distribution of elemental mercury is identical to the distribution of mercury ion, with high concentrations in the kidney and the liver, in the intestinal mucosa and in the testicles (Berlin, 1979).
Mercury ion is very rapidly distributed in the tissues where it is found a few hours after exposure. It accumulates in the kidneys, in the liver, in the myocardium, in the mucosa of the intestines, of the upper airways, of the mouth and especially of the nose, in the interstitial tissues of the testicles, in the skin, in the bone marrow and in the placenta.
Accumulation is most marked in the kidney : the quantity of mercury in the renal cortex already exceeds the quantity in the blood one hour after administration. The biological half-life of inorganic mercury accumulated in the kidney, which has been calculated as 64 days (Miettinen, 1971; Katzantzis et al., 1976), is longer than in the whole body (58 days) (Hursh et al., 1976), but in any case shorter than in the central nervous system, where it is as much as a year (Berlin and Hulberg, 1966, I and II; Takahata et al., 1970), but where the uptake is, however, much slower.

There are, however, **considerable differences between organic mercury compounds and inorganic mercury salts as far as the respective concentrations in the CNS are concerned, even if the type of distribution has been confirmed to be the same on the basis of studies of autopsy material from subjects who died following mercury intoxication in Minamata** (Brown and Kulkarni, 1967; Davis et al., 1974; Takahata et al., 1970).

The alkyl compounds cross the blood-brain barrier more easily than the inorganic compounds (Cassitto and Gilioli, 1980).
Further, among the same organic compounds, considerable differences in velocity of removal from tissues and in brain concentrations are found between alkyl and aryl salts. Phenyl mercury, for example, is less rapidly metabolized to inorganic mercury than methoxyethyl mercury (Daniel et al., 1971; Gage, 1975).
Among the alkyl salts, methyl mercury is much more easily transported across the placental barrier, reaching concentrations in the foetal red blood cells 30% higher than those of the mother. **All mercury compounds can be directly or indirectly converted to methyl mercury in the body.** Although such conversions are only seen experimentally, nevertheless the finding of increased organic mercury levels in the urine, in concomitance with an increased exposure in subjects exposed to mercury vapours, shows that this may also happen in the human body (Suzuki and Shishido, 1971).
On the other hand, the possibility of a biotransformation of methyl mercury compounds, with liberation of inorganic mercury, has been demonstrated in several animal species (Nordberg and Skerfving, 1971) and also in humans (Sumino, 1978; Bakir et al., 1973).
Since it has been established that absorption by the intestinal route is 100%, and by evaluating the biological half-life at 70 days, the theoretical accumulation curve of methyl mercury in humans can be calculated for various doses of methyl mercury (Kitamura et al., 1976).

Excretion

Inorganic mercury, by whichever route it has been absorbed, is mainly excreted via the kidneys. A minor degree of excretion occurs through the bile, the intestinal mucosa, the sudorific and salivary glands, the hair and nails, the faeces, and via the skin both by volatilization and desquamation (Swensson et al., 1959; Weed et al., 1962; Berlin, 1963; Magos, 1968). The kidney acts as a multicompartmental model in which at least one compartment is characterized by a high retention time (Miettinen, 1971). However, the exact mechanism of mercury uptake by the plasma and its subsequent release in the tubules are not clear, although experimental research suggests that mercury might be secreted from the kidney tubules. Similarly it has not been established whether the excretion and reabsorption sites are the same, with different pH or mercury concentration gradients, or separate (e.g. excretion through the proximal tubule, reabsorption through the distal tubule).
In any case, it is certain that two processes, excretion and deposition, through the kidney parenchyma, do not proceed in a synchronized manner.

From the onset of exposure there is a latency period in maximum excretion until the kidney has accumulated a certain quantity.

In intermittent exposures (as is the case in many occupational exposures), this latency mechanism can give rise to the appearance of an excretion peak during period of non-exposure.
The persistence of urinary elimination of mercury even for a considerable period of time after cessation of exposure could warrant the supposition that the metal is incorporated irreversibly into the protein cells, after which the excretion frequency would depend on the metabolic turnover of the proteins (Vostal and Heller, 1968).

Comparing kidney accumulation and excretion, quantitative differences can be noted according to the various compounds. With inorganic mercury, 50% of the dose is accumulated in the kidney, less than 10% is excreted, while only 10% of methyl mercury is accumulated in the kidney with an excretion rate 10 times lower than inorganic mercury. About 90% of organic mercury is excreted by the fecal route (Berlin, 1979). Also, a fair portion of mercury is eliminated with perspiration. Even though this route of elimination has not been the subject of much study, it should be noted how in 3 workes at a chlor-alkali plant (Lovejoy et al., 1973) the concentrations of mercury found in the sweat were between 50 and 200% higher than those in the urine. This finding assumes importance if it is considered that high temperatures (with consequent excessive sweating) are frequent in chlor-alkali plants, which are not built in the open.

Biological Monitoring

In view of their different meanings, the biological indicators for inorganic and organic compounds will be dealt with in separate chapters.

Inorganic Compounds

Indicators of internal dose

Mercury in urine

The mean levels of mercury in urine in non-exposed populations resulting from the latest studies were below 10 μg/l even though with rather wide ranges (from 0 to 42 μg/l) and with variations influenced by the area of residence (Kubasik et al., 1972; Cigna Rossi et al., 1976; Stopford et al., 1978; Lie et al., 1982). In occupationally exposed subjects, the urinary mercury levels showed rather conspicuous per cent variations, both from day to day and during the same day, even though the exposure conditions were constant (Goldwater et al., 1963; Piotrowsky et al., 1975).
It was, however, found that the intraindividual variations can be reduced by collecting the urine samples at the same time in the morning (Piotrowsky, 1975).
However, according to Stopford et al. (1978), urinary mercury at the end of the working week is as indicative of mean exposure to mercury vapours in that week as the mean of the urinary mercury values in samples collected over the entire week.

After removal of subjects from exposure, two phases of urinary excretion of mercury were identified : the first phase lasts on average 2 days and accounts for not more than 20-30% of excretion in steady state; the second phase has a half-life of 70 days (Piotrowsky et al., 1975). This confirms the experimental data obtained in human subjects with marked mercury, showing that mercury has a half-life of 64 days in the kidney (Cherian, 1978) and is indicative of the tendency of mercury to accumulate in the kidney until steady state is reached, i.e. saturation of the binding sites in the tissue (metallothioneine). In a subject exposed to constant environmental mercury concentrations, this state is reached in approximately one year; before this time, the urinary mercury values are thought to be more indicative of the quantity of metal deposited in the kidney rather than of the current environmental exposure levels (Stopford, 1978). This explains why in intermittent exposures (as is the case in many occupational exposures) high urinary excretions can occur during a period of non-exposure.
The persistence of urinary elimination of mercury, even for a considerable period of time after cessation of exposure, has also been explained by a mechanism of irreversible incorporation of the metal into the cellular proteins of the kidney; the persistence of mercury excretion is believed to depend on the metabolic turnover of the proteins (Vostal and Heller, 1968).
There is rather unanimous agreement among the various studies that the urinary mercury levels, when mean group values are considered, are well correlated with the mean atmospheric concentrations of mercury at the workplace. In workers of a chlor-alkali plant, Smith et al. (1968) found urinary group values between 100 and 300 μg/l for environmental mercury concentrations of 0.1 mg/m^3; the urinary values fell by 50% at environmental concentrations of 0.05 mg/m^3. In a later study, the same author established a ratio of 1.25 between urinary and environmental mercury on a group basis (Fig. 2)

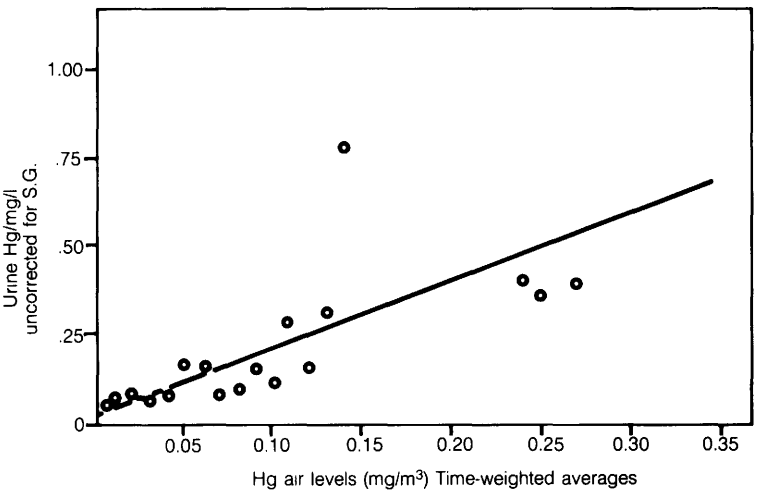


Fig. 2 Relationship between mean mercury concentrations in air and mean values of HgU in groups of chlor-alkali workers.
(From Smith et al., 1970)

Lauwerys et al. (1973), again for group values, suggested that for exposures to 0.05 mg/m^3 (ACGIH-TLV), the urinary values are about 50 μg/l rather than 100 μg/l. A 1/1 ratio was found in other studies (Bell, 1973; Nakaaki, 1975; Stopford et al., 1978; Gambini, 1978) (Fig. 3). The WHO Study Group (1979) accepted a ratio of 1/2 as more likely.

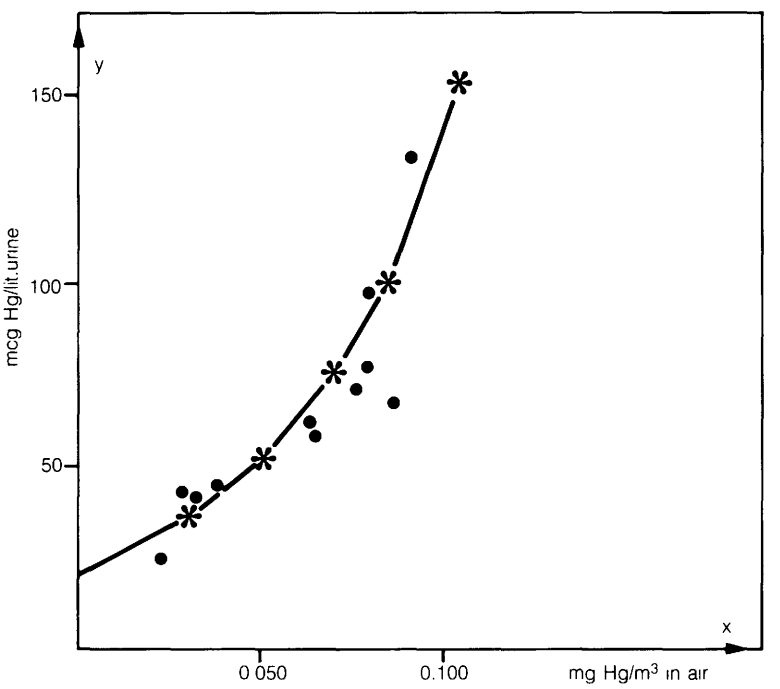


Fig. 3 Relationship between mean concentrations of mercury in air and mean values of HgU in groups of chlor-alkali workers.
(From Gambini, 1978)

The possibility that a correlation between individual urinary mercury levels and environmental mercury levels could exist was revealed by the studies of Bell (1973) and Stopford et al. (1978), who assigned greater importance to the microenvironment (contamination of hands and clothing) as a significant source of mercury vapours. In particular, Stopford et al. (1978), using personal samplers, found a close correlation between urinary mercury and atmospheric mercury present in the microenvironment, while only a moderate correlation was found between urinary mercury and atmospheric mercury concentrations in the macro-environment.

Gambini (1978) did not find any significant increase in psychic erethismus and tremor in exposed subjects when urinary mercury values were below 50 μg/l. The WHO tentatively proposed 50 μg/l as a limit value since, on the basis of a 1/1 ratio, this corresponds to an atmospheric value of 0.05 mg/m^3, which is the ACGIH's TLV-TWA value (1984). The WHO changed this value to 50 μg/g creatinine in 1985 (individual level).

Landolf et al. (1978) did not find any alterations in psychometric tests or tremour measurement tests in exposed subjects whose urinary mercury values had not exceeded 50 μg/l in the previous year.
An increased frequency of abnormalities in psychometric tests and tremometry was found when urinary mercury values were between 50 and 100 μg/g creatinine (Roels et al., 1982). At these values there was also an increased frequency of proteinuria and albuminuria compared to a control group (Buchet, 1980; Roels, 1982).

It should, however, be emphasized that even if HgU can be considered as an exposure indicator, it cannot be used to assess the dose in the critical organ, i.e. the brain, because the average life of mercury deposited in the brain is much longer than in other organs and therefore high quantities can remain in the brain after cessation of exposure.

Mercury in total blood

In the most studies on populations consuming moderate or negligible quantities of fish, the mean values of mercury in blood in non-occupationally exposed subjects were below 3 μg/100 ml, with a range from 0.03 to 10 μg/100 ml (Paccagnella et al., 1973; Turner, 1974; Den Tonkelar, 1974; Cigna Rossi et al., 1976; Bourcier et al., 1982). Few data are available on blood mercury levels in exposed subjects. Smith et al. (1970) found values below 5 μg/100 ml over a total of 117 individuals.

Lauwerys et al. (1973) found mean values of 0.65 ± 0.11 μg/100 ml over a total of 23 subjects, whereas Roels et al. (1982) found mean values of 0.23 μg/100 ml with a range of 0.05 - 0.59 over a total of 47 individuals.

Significant correlations between blood mercury levels and atmospheric mercury were found by Smith et al. (1970) on a group value basis.
Stopford et al. (1978) found a significant correlation between blood mercury values and mean values of microenvironmental exposure measured on the previous five days, but not with the microenvironmental exposure values measured on the day blood samples were taken for HgB assessment.
Lindstedt et al. (1979) too found a significant correlation between individual mercury levels in blood in the middle of the week and mean atmospheric mercury levels of the previous week. The authors calculated that for atmospheric values of 50 μg/m^3, the corresponding blood mercury values were 30-35 μg/l, in agreement with the findings of Smith et al. (1970), who calculated that for environmental values of 0.10 mg/m^3, the corresponding values of blood mercury were 60 μg/l (group values).

There was a good correlation between urinary and blood mercury in the study made by Smith et al. (1970), considering group values; a ratio of 0.3 was established between the two variables.
However, other studies did not find any correlationship between the two parameters (Stopford et al., 1978; Lindstedt et al., 1982).
Stopford et al. (1978) suggested that the relationship between urinary and blood mercury could in some way be influenced by the length of exposure and by the different kinetic behaviour of the two compartments, even though blood mercury reflects a part of the tissue deposits, as demonstrated by the finding of high values of mercury in blood in subjects no longer exposed even for six years (Goldwater and Nicolau, 1966).

No limit values for mercury in blood have been proposed for occupationally exposed subjects. A value of 1 μg/100 ml was proposed as a "Health evaluation action level" (Miller et al., 1975) which should offer protection against subclinical alterations of the central nervous system.
An increased frequency of abnormalities in psychometric tests at blood mercury levels between 1 and 2 μg/100 ml was found by Roels et al. (1982).
The results of an experimental study on humans with marked mercury suggest that the levels of mercury in blood could be indicators of recent exposure, as distinct from urinary mercury, which could be indicative of renal accumulation (Cherian et al., 1978). Moreover, the finding of high levels of mercury in blood in subjects no longer exposed for as long as six years suggests that a relationship nontheless exists between blood mercury and tissue deposits of mercury (Goldwater and Nicolau, 1966).

Indicators of effect

In general terms, it should be emphasized that some symptoms, such as anorexia, weight loss, tremor, insomnia, were well correlated with the degree of exposure, while other symptoms were less correlated (Smith, 1970).
As far as specific indicators of effect are concerned, it can be said that **at the present time, no biological tests can be identified which will permit biological monitoring of the exposed individual, especially in relation to possible early biochemical lesions, when the alterations are still reversible.**

Controversy exists, however, when a group of exposed workers is under consideration. In fact, epidemiological research has, for example, shown that significant correlations exist between mercury exposure and inhibition of erythrocyte cholinesterase (Wada et al., 1969; Lauwerys and Buchet, 1973). Nevertheless, the data reported in the literature do not agree as to the part played by the erythrocyte cholinesterases (Foà et al., 1976).

There has also been evidence of inhibition of membrane (Na^+K^+)- ATPase of RBCs with increase in blood and urinary LDH, especially fractions 4a and 5a (Singerman and Catalina, 1971).

The wide dispersion of individual results in fact precludes using the measurement of the above enzymes as individual indices of early effects due to inorganic mercury absorption. Interest has been aroused by some investigations on populations occupationally exposed to mercury vapours, which have confirmed previous statements concerning selectivity of accumulation of Hg at the lysosomal level. Significant increases in the plasmatic activity of some lysosomal acid hydrolases (β-glucosidase, β-galactosidase, β-glucoronidase, N - acetyl glucosaminidase) were found in subjects exposed even to concentrations below 50 $\mu g/m^3$ (Lauwerys and Buchet, 1973; Foà at al., 1976).

As already mentioned, during chronic exposure to elemental and inorganic mercury, the critical organ is the central nervous system, involvement of which is confirmed by various symptoms but above all by alterations in psychomotorial performance (Skerfving and Vostal, 1972). Attempts have been made to evaluate the early signs of these changes in exposed persons in relation to dose indices.
The tests used include psychometric tests and intellectual function and personality analysis of exposed subjects.
A significant correlation was found between performance in the psychometric tests and urinary mercury (Chaffin et al., 1973). Also the results of mechanical and visual memory tests and psychometric ability and personality tests were well correlated with the number of times in a year over the last 10 years in which urinary mercury had exceeded 100 $\mu g/l$ (Forzi et al., 1976). Similarly, the results of psychometric tests were correlated with the number of urinary mercury peaks above 0.5 mg/l observed in the previous, 3, 6 and 12 months. Angotzi et al. (1980) found significant differences in perceptive intelligence, psychometric ability and memory tests and in personality tests (especially those on socio-emotional balance) in a group of subjects who had had urinary mercury levels exceeding 50 $\mu g/l$ for 3 times in a year, compared with a control group.

The neurophysiological studies made by Chaffin et al. (1974) and Langolf et al. (1978) showed a reduction in electromyographic frequencies towards lower levels that was correlated with the increase in urinary mercury levels.
The same authors reported an increase in the mean tremour frequencies (studied by means of spectral analysis) that was correlated with urinary mercury levels.
A good correlation was found between alterations in electrophysiological parameters (especially velocity conduction) and risk index calculated on the basis of environmental mercury concentrations and length of service in workers of a chloroalkali plant after prolonged exposures. The electrophysiological alterations were well correlated with the objective signs and symptoms of involvement of the motor neurones (Gilioli et al., 1976). The same study also revealed an earlier appearance of the clinical signs of involvement of sensitive nerve fibres.

Organic Compounds

Indicators of Dose

Mercury in blood

Extremely few data are available on the concentrations of mercury in blood in workers exposed to organic mercury compounds.
In a study by Goldwater (1973) on 7 workers exposed for six weeks to methyoxyethyl mercury chloride, mean mercury in blood values of 65 $\mu g/100$ ml (range 34-109 $\mu g/100$ ml) were found, without any clinical signs or symptoms.

Almost all data on mercury in blood in subjects exposed to organic mercury compounds concern non-occupational situations.

A large mass of data were collected on occasion of collective intoxications occurring in the general population caused by consumption of organic mercury compounds with food. In populations of fishermen who consumed fish containing methyl mercury (Minamata) blood mercury levels up to 60 μg/100 ml were found without any signs or symptoms that could be referred to mercury intoxication (Birke, 1973; Skerfving, 1974; Clarkson et al., 1976).

In populations intoxicated with cereals treated with organic mercury pesticides, a significant correlation was found between mercury in blood and frequency of symptoms of paresthesia that was identical to the correlation found between the latter and body burden.

The concentration threshold of mercury in blood at which paresthesia occurs to a degree above the base level was calculated to be between 24 and 48 μg/100 ml (Bakir et al., 1973). From the data obtained in the population of Niigata, the threshold of appearance of symptoms was found to be between 20 and 40 μg/100 ml (WHO, 1976). No similar studies are available for occupationally exposed subjects.

Mathematical extrapolation was used in order to transpose the data on collective intoxications to the occupational field. It was calculated that the earliest clinical effects, i.e., paresthesia, occur at levels of 5 μg/kg body weight per day in the case of consumption with food. On the basis of a daily volume of 10 m^3 of inhaled air at the workplace for 225 working days, with an 80% retention of mercury products, the TLV that should correspond to such consumption levels would be 0.07 mg/m^3 (WHO, 1976).

Mercury in urine

Very small amounts of MeHg are excreted in the urine and the concentration in urine is easily masked by the inorganic mercury present. Thus, mercury in urine is not a good indicator of internal dose for MeHg (Berlin, 1979).

Mercury in the hair

The hair is an extremely useful biological material for evaluation of exposure to organic compounds. Methyl mercury concentrates in the hair at a much higher rate than in the body or in the blood. A linear correlation was established between total mercury levels in blood and mercury in hair levels, with ratios from 1:300 (Berglund et al., 1971; Nordberg and Skerfving, 1971) to 1:500 (Tsubaki, 1971). Hair levels can therefore be used as an indicator of "dose" in the whole body as well as in the brain, and are consequently an invaluable parameter in the surveillance of exposed general populations. However, since hair grows at different rates and can obviously be subject to external contamination, caution must be used in evaluating this parameter. **For this reason, since external contamination is very common in working environments and since mercury can bind stably with the keratine-SH groups, this method is not suitable for monitoring occupational exposure.** Nevertheless, the sequential estimation of mercury values in segments of hair measuring 2.5 cm taken from women involved in the epidemic in Iraq (Katzantzis et al., 1976) has provided information on the period of mercury accumulation, which went back to one year before the hair samples were taken.

Conclusions

The metabolism and toxicology of mercury vapours and inorganic mercury compounds differ substantially compared to organic compounds, to which, moreover, the general population is mainly exposed. Also, the meaning of the biological indicators differs according to whether exposure was to inorganic or organic mercury.

For subjects occupationally exposed to mercury vapours and inorganic mercury the test most widely used as an indicator of internal dose is HgU, the levels of which, at least on a group basis, are correlated with the environmental concentrations. In the most recent studies reported, a ratio of 1:1 was established between HgAir measured with personal samplers and HgU in μg/l. The HgU levels are used as indicators of exposure after a latency period of between 6 months and one year from the beginning of exposure. After cessation of exposure the HgU levels may fall progressively; however, in the brain (the critical organ) the mercury levels remain high for a long period of time (years).

Fewer data are available on the reliability of HgB as an indicator of exposure since very few studies have been carried out on the subject.
In some studies a good relationship was found between HgU and HgB values. In exposure to organic mercury compounds, the only test generally accepted as valid for biological monitoring is HgB, which is a complete indicator of exposure for working populations that can integrate the quantity of mercury absorbed by inhalation with the quantity absorbed through the skin which, in the case of organic compounds, is one of the main routes of absorption. HgU, however, is of very little value since organic mercury is eliminated with urine only to a very limited extent.
In the present state of knowledge, no indicators are available that are capable of revealing a reversible biological effect either for exposure to mercury vapours or to organic mercury compounds.

Needs for Further Research

The following are recommendations for further research, for both organic and inorganic mercury exposures :

- evaluation of the relationship between HgAir and HgU and HgB, measuring HgAir with personal samplers
- study of the behaviour of HgB and HgU in relationship to duration of exposure, and evaluation of the relationship between HgB, HgU and integrated exposure (dose x time)
- study of the relationship between the indicators of internal dose HgU and HgB and :
  - lysosomal-derived plasmatic enzymes
  - behavioural test
  - test of early neurological damage.

References

ACGIH: Threshold limit values for chemicals substances in work air adopted by the American Conference of Governmental Industrial Hygienist for 1984, Cincinnati (1984)

AL-DAMLUJI S.F. (1976) The Clinical Committee on Mercury Poisoning. In: Conference on Intoxication due to Alkylmercury Treated Seed 1971-72 Outbreak in Iraq - Bagdad 1974. **WHO Suppl. Vol. 53,** pp. 65-81, Geneva 1976

ANGOTZI G., CASSITTO M.G., CAMERINO D., CIONI R., DESIDERI E., FRANZINELLI A., GORI R., LOI F., SARTORELLI E. (1980) Rapporti tra esposizione a mercurio e condizioni di salute in un gruppo di lavoratori addetti alla distillazione di mercurio in uno stabilimento della provincia di Siena. **Med. Lav. 71**, 463-481

BAKIR F., DAMLUJI S.F., AMIN—ZAKI L., MRTHADA M., KHALIDI A., AL-RAWI N.Y., TIKRITI S., DAHIR H.I., CLARKSON T.W., SMITH J.C., DOHERTY R.A. (1973) Methylmercury poisoning in Iraq. **Science 181**, 230-241

BALDI G., VIGLIANI E.C., ZURLO N. (1953) Mercurialismo cronico nei cappellifici. **Med. Lav. 44**, 161

BELL Z.G., LOVEJOY H.B., VIZENA T.R. (1973) Mercury exposure evaluations and their correlations with urine mercury excretion. 3. Time-weighted average (TWA) mercury exposure and mercury urine levels. **J. Occup. Med. 15**, 501

BERGLUND F., BERLIN M., BIRKE G., CEDERLOF R., VON EULER U., FRIBERG L., HOLMSTEDT B., JONSSON E., LUNING K.C., RAMEL C., SKERFVING S., SWENSSON A., TEJNING S. (1971) Methylmercury in fish: A toxicologic-epidemiologic evaluation of risks. Nord. **Hyg. Tidskr., suppl. 4**

BERLIN M. (1963) Renal uptake, excretion and retention of mercury. II. A study in the rabbit during infusion of methyl and phenyl mercuric compounds. **Arch. Environ. Health 6**, 626

BERLIN M. (1979) Dose-response relations and diagnostic indices of mercury concentrations in critical organs upon exposure to mercury and mercurials. In: Effects and Dose-Response Relationships of Toxic Metals, Nordberg G.F. (Ed.), Elsevier Scientific Publishing Company, Amsterdam, pp. 235-245

BERLIN M., FAZACKERLY J., NORDBERG G. (1969) The uptake of mercury in the brains of mammals exposed to mercury vapor and to mercuric salts. **Arch. Environ. Health 18**, 719

BERLIN M., JERKSELL L.G., VON UBISCH H. (1966) Uptake and retention of mercury in the mouse brain. A comparison of exposure to mercury vapor and intravenous injection of mercuric salts. **Arch. Environ. Health 12**, 33-42

BERLIN M., ULLBERG S. (1963) Accumulation and retention of mercury in the mouse. I. An autoradiographic study after a single intravenous injection of mercuric chloride. **Arch. Environ. Health 6**, 589

BERLIN M., ULLBERG S. (1963) Accumulation and retention of mercury in the mouse. II. An autoradiographic comparison of phenylmercuric acetate with inorganic mercury. **Arch. Environ. Health 6**, 602

BIRKE G., JOHNELS A.G., PLANTIN L.O., et al. (1972) Studies of humans exposed to methyl mercury through fish consumption. **Arch. Environ. Health 25**, 77

BOURCIER D.R., SHARMA R.P., DROWN (1982) A stationary cold vapor method for atomic absorption measurement of mercury in blood and urine used for exposure screening. **Am. Ind. Hyg. Ass. J. 43**, 329

BROWN J.R., KULKARNI M.V. (1967) A review of the toxicity and metabolism of mercury and its compounds. **Med. Serv. J. Can. 23**, 786-808

BROWNING E. (1969) Toxicity of industrial metals. London

BUCHET J.P., ROELS H., BERNARD A., LAUWERYS P. (1980) Assessment of renal function of workers exposed to inorganic lead, cadmium or mercury. **JOM 22**, 241-250

CASSANO G.B., VIOLA P.L., GHETTI B., AMADUCCI L. (1966) The distribution of inhaled mercury (Hg-203) vapours in the brains of rats and mice. **J. Neuropathol. Exp. Neurol. 28**, 308

CASSITTO M.G.., GILIOLI R. (1980) Mercurio e sistema nervoso. **G. Ital. Med. Lav. 2**, 181-186

CHAFFIN B., MILLER M. (1974) Behavioral and neurological evaluation of workers exposed to inorganic mercury. In: Xintaras C. (ed.) Behavioral toxicology. pp. 297-313. National Institute for Occupational Safety and Health, Washington D.C.

CIGNA-ROSSI L., CLEMENTE G.F., SANTARONI G. (1976) Mercury and selenium distrubution in a defined area and in its population. **Arch. Environ. Health 31**, 160-165

CLARKSON T.W. (1971) Epidemiological and experimental aspects of lead and mercury contamination of food. **Food Cosmet. Toxicol. 9**, 229

CLARKSON T.W. (1972) The pharmacology of mercury compounds. In: Annual Review of Pharmacology, **12**, 375-406. Elliot H.W. (ed.), Palo Alto, Annual Reviews, Inc.

CLARKSON T.W. (1972) Recent advances in the toxicology of mercury with emphasis on the alkylmercurials. C.R.T. **Crit. Rev. Toxicol. I**, 203

CLARKSON T.W., GATZY J., DALTON C. (1961) Studies on the equilibrium of mercury vapor with blood. Division of Radiation Chemistry and Toxicology, University of Rochester, Atomic Energy Project, Rochester, New York, UR-582, p. 64

CLARKSON T.W., MARSH D.O. (1976) The toxicity of methylmercury in humans: dose-response relationships in adult populations. In: Effects and Dose-Response Relationships of Toxic Metals, Nordberg G.F. (ed.), pp. 246-261, Elsevier Scientific Publishing Company, Amsterdam

DANIEL J.W., GAGE J.C., LEFEVRE P.A. (1971) The metabolism of methoxyethylmercury chloride in the rat. **Biochem. J. 121**, 411-415

DAVIS L.E., WANDS J.R., WEISS S.A., PRICE D.L., GIRLING E.F. (1974) Central nervous system intoxication from mercurous chloride laxatives. Quantitative, histochemical and ultrastructural studies. **Arch. Neurol. (Chic.) 30**, 428-431

DEN TONKELAAR E.M., VAN ESCH G.J., HOFMAN B. (1974) In: Proceedings of an International Symposium on Recent Advances in the Assessment of the Health Effects of Environmental Pollution. Vol. 2, pp. 1017-1027, Paris, CEC Luxembourg

EUROSTAT : EC raw materials balance sheets 1979-1982. p. 180. Office for official publications of the European Communities, Luxembourg, 1985

FOA' V., CAIMI L. (1977) Patologia da mercurio e suoi composti. In: Trattato di medicina del lavoro, Sartorelli E. (ed.), Piccin Editore, Padova, pp. 353-376

FOA' V., CAIMI L., AMANTE L., ANTONINI C., GATTINONI A., TETTAMANTI G., LOMBARDO A., GIULIANI A. (1976) Patterns of some lysosomal enzymes in the plasma and of proteins in urine of workers exposed to inorganic mercury. **Int. Arch. Occup. Environ. Health 37**, 115-124

FORZI M., CASSITTO M.G., BULGHERONI C., FOA' V. (1976) Psychological measures in workers occupationally exposed to mercury vapors. A validation study. In: Adverse effects of environmental chemicals and psychotropic drugs, Vol. 2, pp. 165-172, Elsevier Amsterdam

FRIBERG L., NORDBERG G.F. (1972) Inorganic mercury. Relations between exposure and effects. In: Mercury in the Environment. A Toxicological and Epidemiological Appraisal, Friberg L. and Vostal J. (eds.), pp. 130-140, CRC Press, Cleveland

FRIBERG L., VOSTAL J. (1975) In: Mercury in the Environment. A Toxicological and Epidemiological Appraisal, CEC Press, Cleveland

GAGE J.C. (1975) **Toxicol. Appl. Pharmacol. 32**, 225-238

GAMBINI G. (1978) Correlazione fra concentrazioni di vapori di mercurio inorganico nell'aria, idrargiruria e sintomi di mercurialismo cronico in un impianto cloro-soda con celle eletrrolitiche a catodo di mercurio. **Med. Lav., Suppl. 3**, 379-392

GILIOLI R., BULGHERONI C., CAIMI L., FOA' V., FILIPPINI C., BOIARDI A., BUSSONE G., QUARTI N., BOERI R. (1976) Correlation between subjective complaints and objective neurophysiological findings in workers of a chloralkali plant. In: Adverse effects of environmental chemicals and psychotropic drugs, Vol. 2, pp. 157-104, Elsevier, Amsterdam

GOLDWATER L.J., LADD A.C., JACOBS M.B. (1964) Absorption and excretion of mercury in man. VII. Significance of mercury in blood. **Arch. Environ. Health 9**, 735-741

GOLDWATER L.J., NICOLAU A. (1966) Absorption and excretion of mercury in man. IX. Persistence of mercury in blood and urine following cessation of exposure. **Arch. Environ. Health 12**, 196

HAMILTON A., HARDY H.L. (1949) Mercury. In: Industrial Toxicology, Hoeber P.B. Inc. Publ., New York, pp. 104-126

HURSH J.B., CLARKSON T.W., CHERIAN M.G., VOSTAL J.J., VANDER MALLIE R. (1976) Clearance of mercury (Hg-197, Hg-203) vapor inhaled by human subjects. **Arch. Environ. Health 31/6**, 302-309

KAZANTZIS G., AL-MUFTI A.W., AL-JAWAD A., AL-SHAHWANI Y., MAJID M.A., MAHMOUD R.M., SOUFI M., TAWFIA K., IBRAHIM M.A., DABAGH H. (1976) Epidemiology of organomercury poisoning in Iraq. Relationship of mercury levels in blood and hair to exposure and to clinical findings. In: Conf. on intoxication due to alkyl-mercury-treated seed, Bagdad, Iraq, 9-13 September 1974, Geneva, WHO, **Bull. of WHO, Suppl. Vol. 53**, 37-48

KUBASIK N.P., SINE H.E., VOLOSIK H.T. (1972) Rapid analysis for total mercury in urine and plasma by flameless atomic absorption analysis. **Clin. Chem. 18**, 132

KITAMURA S., SUMINO K., HAYAKAWA K., SHIBATA T. (1976) Dose-response relationship of methylmercury. In: Effects and Dose-Response Relationships of Toxic Metals, Nordberg G.F. (ed.), Elsevier Scientific Publishing Company, Amsterdam, pp. 262-272

LANGOLF G.D., CHAFFIN D.B., HENDERSON R., WHITTLE H.P. (1978) Evaluation of workers exposed to elemental mercury using quantitative tests of tremor and neuromuscolar function. **Am. Ind. Hyg. Assoc. J. 39**, 976-984

LAUWERYS R., BUCHET J.P. (1973) Occupational exposure to mercury vapors and biological action. **Arch. Environ. Health 27**, 65-68

LEVINE S.P., CAVENDER G.D., LANGOLF G., ALBERS J.W. (1982) Elemental mercury exposure; peripheral neurotoxicity. **Brit. J. Ind. Med. 39**, 136-139

LIE A., GUNDERSEN N., KORSGAARD K.J. (1982) Mercury in urine. Sex, age and geographic differences in a reference population. **Scand. J. Work. Environ. Health 8**, 129-133

LINDSTEDT G., GOTTBERG I., HOLMGREN B., JONSSON T., KARLSSON G. (1979) Individual mercury exposure of chloroalkali workers and its relation to blood and urinary mercury levels. **Scand. J. Work. Environ. Health 5**, 59-69

LOVEJOY H.B., BELL Z.G., VIZENA T.R. (1973) Mercury exposure evaluations and their correlations with urine mercury excretion. IV. Elimination of mercury by sweating. **J. Occup. Med. 15**, 590

MAGOS L. (1967) Mercury-blood interaction and mercury uptake by the brain after vapor exposure, **Environ. Res. I**, 323

MAGOS L. (1968) Uptake of mercury by the brain. **Br. J. Ind. Med. 25**, 315

MAGOS L., SUGATA Y., CLARKSON T.W. (1974) Effects of 3-amino-1,2,4-triazole on mercury uptake by *in vitro* human blood samples and by whole rats. **Toxicol. Appl. Pharmacol. 28**, 367-373

MIETTINEN J.K. (1971) Absorption and elimination of dietary mercury (Hg^{+2}) and methyl-mercury in man. In: Mercury, Mercurials and Mercaptans, Miller M.W. and Clarkson T.W. (eds.), Charles & Thomas Publisher, Springfield, Ill. (U.S.A.), 233-243

MILLER J.M., CHAFFIN D.B., SMITH R.G. (1975) Subclinical psychomotor and neuromuscular changes in workers exposed to inorganic mercury. **Am. Ind. Hyg. Assoc. J. 36**, 725-733

NAKAAKI K. (1975) On biological evaluation of inorganic mercury exposure by urinary levels. Communication at the XVIII Internat. Congr. Occup. Health, Brighton (U.K.), 14-19 September 1975

NIELSEN-KUDSK F. (1971) Biological oxidation of elemental mercury. In: Mercury, Mercurials and Mercaptans, Miller M.W. and Clarkson T.W. (eds.), Charles & Thomas Publisher, Springfield, Ill. (U.S.A.), 355-371

NORDBERG G.F., SKERFVING S. (1971) Metabolism. In: Mercury, Mercurials and Mercaptans, Miller M.W. and Clarkson T.W. (eds.), Charles & Thomas Publisher, Springfield, Ill. (U.S.A.) 29-92

PACCAGNELLA R., PRATI L., BIGONI A. (1973) In: Proceedings of the international symposium on the problems of contamination of man and his environment by mercury and cadmium, CEC, Luxembourg, 1973, pp. 463-479

PIOTROWSKI J.K., TROJANOWSKA B., MOGILNICKA E.M. (1975) Excretion kinetics and variability of urinary mercury in workers exposed to mercury vapours. **Int. Arch. Occup. Environ. Health 35**, 245-256

POPESCU H.I., NEGRU L., LANCRANJAN I. (1979) Chromosome aberration induced by occupational exposure to mercury. **Arch. Env. Health 34**, 461-463

RAHOLA T., HATTULA T., KOROLAINEN A., MIETTINEN J.K. (1973) **Ann. Clin. Res. 5**, 214

ROELS H., LAUWERYS R., BUCHET J.P., BERNARD A., BARTHELS A., OVERSTEYNS M., GAUSSIN J. (1982) Comparison of renal function and psychomotor performance in workers exposed to elemental mercury. **Int. Arch. Occup. Environ. Health 50**, 77-93

SEATON A., BISHOP C.M. (1978) Acute mercury pneumonitis. **Brit. J. Ind. Med. 35**, 258

SINGERMAN A., CATALINA R.L. (1971) Exposure to metallic mercury : enzymatic studies. In: Proceedings of XVI Int. Congr. Occup. Health, 554-557

SKERFVING S. (1972) Organic mercury compounds. Relation between exposure and effects. In: Mercury in the Environment. A Toxicological and Epidemiological Appraisal, Friberg L. and Vostal J. (eds.), CRC Press, Cleveland, Ohio (U.S.A.) 141-168

SKERFVING S., VOSTAL J. (1972) Symptoms and signs of intoxication. In: Mercury in the Environment. A Toxicological and Epidemiological Appraisal, Friberg L., Vostal J. (eds.), CRC Press, Cleveland, Ohio (U.S.A.) 93-108

SKERFVING S., HANSSON K., MANGS C., LINDSTON J., RYMAN N. (1974) Methylmercury-induced chromosome damage in man. **Environ. Res. 7**, 83-98

SKERFVING S., COPPLESTONE J.F. (1976) Poisoning caused by the consumption of organomercury-dressed in seed in Iraq. **WHO 54**, 101-112

SMITH R.G. (1968) Studies of workers exposed to mercury in chlorine industry. Working paper of the Symposium on MAC values, Stockholm (Sweden)

SMITH R.G., VORWALD A.J., PATIL L.S., MOONEY T.F. (1970) Effects of exposure to mercury in the manufacture of chlorine. **Am. Ind. Hyg. Assoc. J. 31**, 687

STOPFORD W., BUNDY S.D., GOLDWATER L.S., BITTIKOFER J.A. (1978) Microenvironmental exposure to mercury vapor. **Am. Ind. Hyg. Assoc. J. 39**, 378-384

SUMINO K.: Proceedings of the Conference on Heavy Metals in the Aquatic Environment, Nashville, Tenn. (U.S.A.), in press

SUZUKI T., SHISHIDO S. (1974) Changes of levels of urinary organic mercury in groups with varying extents of inorganic mercury exposure. **Tohoku J. Exp. Med. 112**, 101

SWENSSON A., LUNDGREN K.D., LINDSTROM O. (1959) Distribution and excretion of mercury compounds after single injection. **Arch. Environ. Health 20**, 432

TAKAHATA N., HAYASHI H., WATANABE B., ANSO T. (1970) Accumulation of mercury in the brains of two autopsy cases with chronic inorganic mercury poisoning. **Folia Psychiat. Neurol. Jap. 24**, 59-69

TEISINGER J., FISEROVA-BERGOROVA V. (1965) Pulmonary retention and excretion of mercury vapours in man. **Ind. Med. Surg. 34**, 580

TSUBAKI T. (1971) Clinical and epidemiological aspects of organic mercury intoxication. In: Proceedings of the Symposium on Mercury in Man's Environment, Ottawa, February 1971, Royal Society of Canada, Ottawa, 131-136

TURNER M.D., MARSH D.O., RUBIO C.E. (1974) In: Proceedings of the First International Congress on Mercury, Barcelona, 1974, Vol. 2, 229-234

VERSCHAEVE L. (1979) Investigation on leukocytes of workers exposed to metallic mercury. **Environ. Mutagen. 1/4**, 259-268

VOSTAL J., HELLER J. (1968) Renal excretory mechanisms of heavy metals. I. Transtubular transport of heavy metal ions in the avian kidney. **Environ. Res. 2**, 1-10

VROOM F.Q., GREER M. (1972) Mercury vapour intoxication. **Brain 95**, 305-318

WADA O., TOYOKAWA K., SUZUKI T. (1969) Response to a low concentration of mercury vapour. Relation to human porphyrin metabolism. **Arch. Environ. Health 19**, 485

WATANABE S. (1969) Mercury in the body ten years after long-term exposure to mercury. In: Proceedings of Int. Congr. Occup. Health, Tokyo (Japan), **14**, 552

WEED R., EBER J., ROTHSTEIN A. (1962) Interaction of mercury with human erythrocytes. **J. Gen. Physiol. 45**, 395

WHO : Baghdad Meeting World Health Organisation Conference on Intoxication Due to Alkylmercury Treated Seed, Baghdad (Iraq) 1974

WHO : Environmental Health Criteria. 1. Mercury. World Health Organisation, Geneva (CH) 1976

WHO : Study Group on Internationally Recommended Health Based Permissible Levels in Occupational Exposure to Heavy Metals. Inorganic Mercury. World Health Organisation, Geneva (CH) 1979

WHO (1985) Internationally recommended health-based occupational exposure limits: a programme in the World Health Organization - El Batawi M.A., Goelzer B.I.M., Ann. Am. Conf. Ind. Hyg. **12**, 49-57

Biological indicators for the assessment of human exposure to industrial chemicals

Organophosphorus Pesticides

M. Maroni

Summary

Organophosphorus (OP) pesticides are widely used as insecticides and herbicides. Exposure concerns manufacturers, formulators, applicators in agriculture and public health, and the general population.

OP pesticides present a serious hazard of acute intoxication. The clinical picture of acute OP poisoining is characterized by signs of cholinergic overstimulation of the central and peripheral nervous system. Fatalities are due to respiratory failure. The mechanism of acute OP poisoning consists of inhibition of acetylcholinesterase (AchE), the enzyme that hydrolyses acetylcholine,at the sites where Ach acts as a neurotransmitter. During acute OP poisoning, a marked inhibition of erythrocyte and serum cholinesterases is also measurable.

Among the chronic effects of OP exposure, the most prominent is a peripheral neuropathy («delayed neuropathy») which may arise few weeks after an acute poisoning or on chronic exposure. The delayed neuropathy is induced only by the limited number of OP pesticides which are able to interact with a specific esteratic enzyme of the nervous tissue, called «Neuropathy Target Esterase» (NTE). Clinically, the delayed neuropathy is a primarily axonal motor neuropathy involving the long nerves of the limbs, which can lead to paralysis. Other chronic effects reported on OP exposure concern the central nervous system (behavioral changes), transient modifications of electromyographic parameters, liver function modifications, and other metabolic changes (microsomal enzyme induction and porphyrin metabolism modification). However all these effects have been poorly characterized and their existence and importance are under question.

Depending on the conditions of exposure, OP pesticides can be absorbed through inhalation or percutaneous penetration. Ingestion results from accidental events, poor hygienic practices or consumption of food contaminated with OP residues.

OP pesticides containing the P = S bond are not active as such and are activated by the organism through oxidation of the thiono group to P = 0. Inactivation of OP esters is performed through hydrolysis of the lateral chain attached to P, oxidative or enzymatic dealkylation, or other hydrolytic reactions: the metabolites originated by these reactions are deprived of anticholinesterase activity and are eliminated with the urine within a few hours.

The assessment of exposure to OP pesticides through biological indicators is fundamental and, in many cases, represents the only reliable way to evaluate OP exposure. The use of biological indicators, however, encounters some practical difficulties, related to the multiplicity of the OP compounds in use, the discontinuous and various modality of pesticide use in different contexts, and the paucity of knowledge and experience on the use of some of these biological indicators.

Among the indicators of internal dose, the urinary excretion of metabolites is the indicator of choice. For a few compounds, including parathion, parathion-methyl and fenitrothion, simple methods measuring phenolic derivatives in urine can be used. For the majority of the OP's such methods are not available and the measurement of alkylphosphates in urine seems to be the most suitable and appropriate procedure. The analytical method for measuring alkylphosphates in urine is rather sophisticated and laborious. Some OP's including malathion, in addition to alkylphosphates give specific urinary metabolites.
The experience of use of indicators of internal dose for the OP compounds in man is very limited and interpretation of the results may be difficult.

The most important and widely used indicator of exposure to OP is the measurement of blood cholinesterase activity. Red blood cells and serum contain two different enzymes, with different specificity and biological variability. The most appropriate for OP exposure monitoring is erythrocyte acetylcholinesterase (AchE). Inhibition of erythrocyte AchE shows

a fair correlation with level of exposure as well as severity of intoxication in exposed subjects. Data on the relationship between AchE inhibition and urinary excretion of OP metabolites are available for very few OP pesticides.

Cholinesterase activity is related only to the acute toxicity of OP esters to the nervous tissue and does not inform about chronic or delayed neurotoxic effects.
Based almost exclusively on experimental animal studies, the determination of «neurophathy target esterase» activity (NTE) in circulating lymphocytes or platelets has been proposed as a test to assess the human risk to develop delayed neurotoxic effects from OP's. This test however is still at an experimental phase and further studies are required to allow its utilization in man.

Very recent investigations have shown that certain OP pesticides may be inducers of liver microsomal enzimes or interfere with liver porphyrin metabolism in **in vitro** systems. If the occurrence of these effects is confirmed also in man, the evaluation of microsomal enzyme induction and the determination of the pattern of urinary or plasmatic porphyrins could be of use as biological indicators of effects in the future.

Organophosphorus Pesticides

Introduction

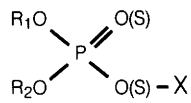
Pesticides occupy a special position among the many chemicals to which man can be exposed, in that they are deliberately spread into the environment for the purpose of killing or injuring some form of life.
Ideally their injurious action should be highly specific for undersirable target organisms and innocuous to desirable, non-target organisms. In fact, however, most of the chemicals in use as pesticides are not highly selective, and organophosphorus (OP) pesticides do not escape this rule. The lack of highly selective pesticidal action represents a risk both for man and other desirable forms of life that coinhabit the environment, thus concern has been growing about the side effects on the environment and human health caused by an unrestricted and uncontrolled use of pesticides.
Organophosphorus esters are one of the most important class of pesticides; they are used as insecticides and, to a lesser extent, as herbicides. Utilization of OP insecticides occurs in agriculture and in public health application for the purpose of controlling vector-borne diseases. Moreover these compounds are freely available on the retail market and are used in great amounts in gardens, green-houses and indoor plant growth by almost the entire population. This greatly enlarges the number of potentially exposed subjects and accounts for the number of fatalities and accidental poisonings worldwide recorded every year. Unlike other industrial, man-made chemicals, exposure to OP pesticides may concern a great part of human population, ranging from the most heavily exposed groups (manufacturers, formulators, professional applicators in agriculture or in public health) to the general population which may experience exposure because of domestic use, proximity to agricultural settings or consumption of contaminated food.
Despite the magnitude of the problem, knowledge about toxicity of OP pesticides in humans is mostly confined to their acute toxic effects. Chronic nervous system damage by these compounds has been only recently recognized and investigation on long-term irreversible effects, such as genetic cell damage or carcinogenicity, is still embrionic. Biological indicators for the evaluation of human exposure to OP's are rather undeveloped and still at an experimental phase. With the exception of the measurement of cholinesterase in red blood cells and serum, there is only a scarce experience of their use and some tests recently developed are not yet applicable on a routine basis because of their complexity and limits of interpretability.
This disappointing picture originates from difficulties which are common to all pesticides. First of all, this class of chemicals comprises numerous different compounds developed and marketed to overcome acquired insect resistance. Since each compound has its own toxic properties and biological behaviour, this variety of chemicals under continuous change makes difficult any acquisitions of consolidated knowledge. Moreover the assessment of the relationship between exposure and absorption, which is essential to allow the use of any biological indicator of dose, is particularly difficult for OP's because many of them are absorbed through the skin and techniques of ambient monitoring adopted in the industrial settings are only very partially usable in agriculture, where the main source of occupational exposure takes place. Finally, while for acute OP toxicity the biochemical target has been identified since long time, investigation about chronic and long-term effects has just started and this prevents from a thorough understanding of the relationship existing between exposure and biological effects. The body of knowledge, however, is rapidly expanding and, as the evaluation of exposure through biological tests tends to be included

in every preventive program for agricultural or industrial workers, improvement and standardization of the methods of monitoring can be expected in a near future.

Chemical and physical properties

Most of the phosphorus-containing insecticides are derivatives of the phosphoric and thiophosphoric acids and can be represented by the general formula shown in Figure 1,

Fig. 1 General structural formula of organophosphorus pesticides.



where R_1 and R_2 are either ethyl (C_2H_5—) or methyl (CH_3—) substituents. Depending on the configuration of the oxigen or sulfur atoms, six main groups of OP's may be distinguished: phosphates, O-phosphorothioates, S-phosphorothioates, phosphorodithioates, phosphonates and phosphoramidates (Fig. 2). Each of these groups comprises many individual compounds. Figures 3 to 7 show the chemical structure and chemico-physical properties of some representatives of each group.

Fig. 2 General structural formula of organophosphorus pesticides.

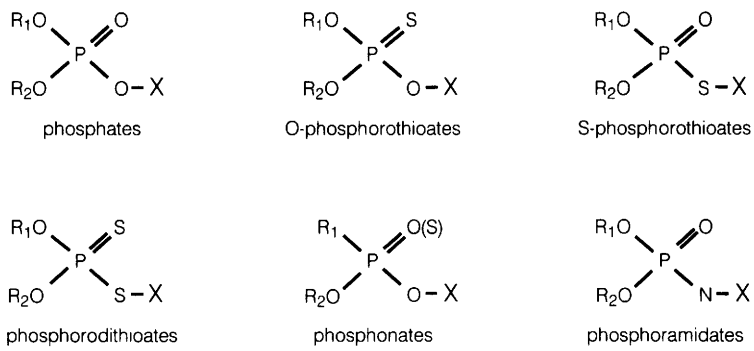


Fig. 3 Names, formula and chemico-physical properties of some phosphates.

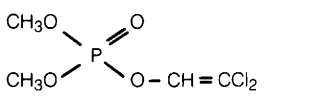
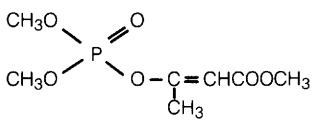
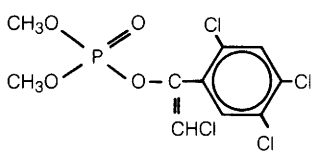
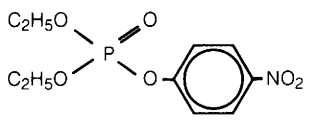
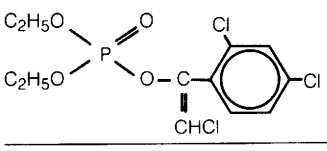
FORMULA	NAMES	PHYSICAL STATUS	SOLUBILITY IN WATER
	**dichlorvos** 0,0-dimethyl,0-(2,2-dichlorovinyl) phosphate	liquid bp 140°	1%
	**mevinphos** 2-methoxycarbonyl-1-methyl-vinyl dimethyl phosphate	yellow liquid cis isomer 100 times more potent	soluble
	**tetrachlorvinphos** 2-chloro-1-(2,4,5-trichlorophenyl)vinyl dimethyl phosphate	solid mp 97 – 98°	11 ppm
	**paraoxon** 0,0-dimethyl-0-p-nitrophenyl phosphate	oily liquid $bp_{1\,0}$ 170°	0.24%
	**chlorfenviphos** 2-chloro-1-(2,4-dichlorophenyl) vinyl dimethyl phosphate	amber liquid	145 ppm

Fig. 4 Names, formula and chemico-physical properties of some phosphorothioates.

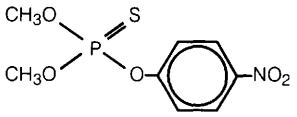
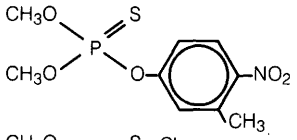
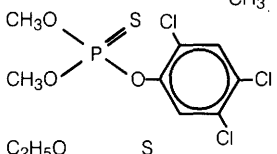
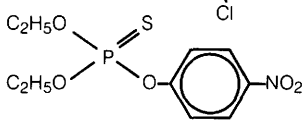
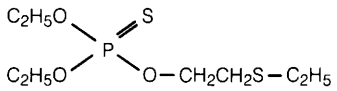
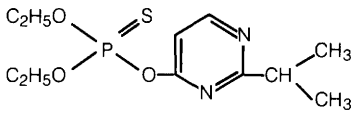
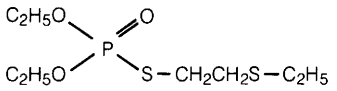
FORMULA	NAMES	PHYSICAL STATUS	SOLUBILITY IN WATER
	**parathion-methyl** 0,0-dimethyl 0-p-nitrophenyl phosphorothioate	crystals mp 37°	50 ppm
	**fenitrothion** 0,0-dimethyl 0-(3-methyl-4-nitrophenyl) phosphorothioate	yellow oil	practically insoluble
	**fenchlorphos** 0,0-dimethyl 0-(2,4,5-trichlorophenyl) phosphorothioate	white powder mp 41°	40 ppm
	**parathion** 0,0-diethyl-0-p-nitrophenyl phosphorothioate	pale yellow liquid bp 375°	20 ppm
	**demeton - 0** 0,0-diethyl 0-[2-(ethylthio)ethyl] phosphorothioate	oily liquid	practically insoluble
	**diazinon** 0,0-diethyl (2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate	liquid with faint ester-like odor	40 ppm
	**demeton - S** 0,0-diethyl S-[2-(ethylthio)ethyl] phosphorothioate	oily liquid	practically insoluble

Fig. 5 Names, formula and chemico-physical properties of some phosphorodithioates.

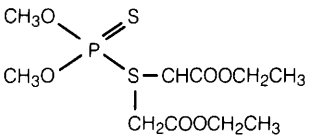
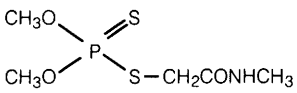
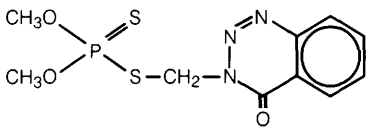
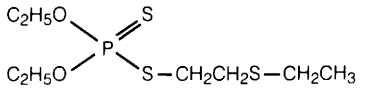
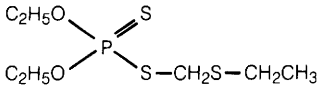
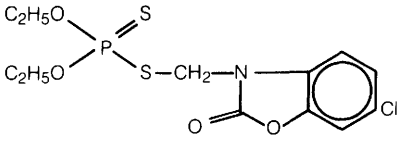
FORMULA	NAMES	PHYSICAL STATUS	SOLUBILITY IN WATER
	**malathion** 0,0-dimethyl S-[1,2-di(ethoxycarbonyl)ethyl] phosphorodithioate	brown to yellow liquid	145 ppm
	**dimethoate** 0,0-dimethyl S-(N-methyl-carbamoylmethyl) phosphorodithioate	crystals mp 52°	very slightly soluble
	**azinphos-methyl** 0,0-dimethyl S-(3,4-dihydro-4-oxobenzo-[1,2,3]-triazin-3-ylmethyl) phosphorodithioate	crystals mp 73°	33 ppm
	**disulfoton** 0,0-diethyl S-[2(ethylthio)-ethyl] phosphorodithioate	colorless oil	insoluble
	**phorate** 0,0-diethyl S-[(ethylthio)-methyl] phosphorodithioate	clear liquid	50 ppm
	**phosalone** 0,0-diethyl S-(6-chlorobenzoxazolon-3-yl)methyl phosphorodithioate	crystals mp 48°	practically insoluble

Fig. 6 Names, formula and chemico-physical properties of some phosphonates.

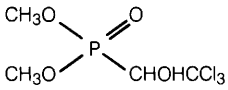
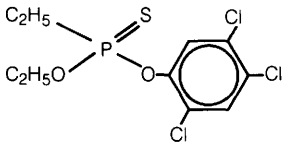
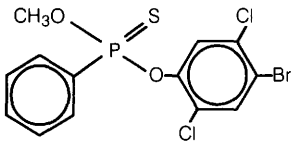
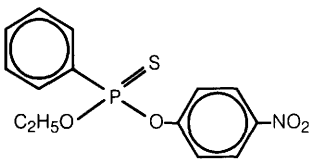
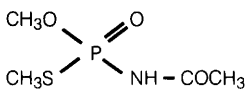
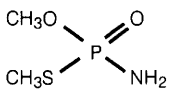
FORMULA	NAMES	PHYSICAL STATUS	SOLUBILITY IN WATER
	**trichlorfon** 0,0-dimethyl (1-hydroxy-2,2,2-trichloroethyl) phosphonate	white crystals mp 83 – 84°	soluble
	**trichloronate** 0-ethyl O-(2,4,5-trichlorophenyl) ethyl phosphonothioate		
	**leptophos** 0-methyl 0-(4-bromo-2,5-dichlorophenyl)phenyl phosphonothioate	waxy solid mp 65 – 67°	practically insoluble
	**EPN** 0-ethyl 0-4-nitrophenyl phenyl phosphonothioate	liquid with aromatic odor	practically insoluble

Fig. 7 Names, formula and chemico-physical properties of some phosphoramidates.

FORMULA	NAMES	PHYSICAL STATUS	SOLUBILITY IN WATER
	**acephate** 0,S-dimethyl N-acetyl-phosphoramidothioate	white solid mp 64 – 68°	soluble
	**methamidophos** 0,S-dimethyl phosphoramidothioate	crystals mp 54°	soluble

Chemical stability of OP esters greatly influences their uses, their environmental persistence and the possibility of toxic effects to living organisms.

One of the most important reactions of OP insecticides is their hydrolysis by water. The attack can take place at either the phosphorus or the alkyl chain and generally this reaction produces the lost of pesticidal action as well as toxicity to humans. Susceptibility to hydrolysis depends largely on the group attached to the phosphorus. Moreover, because P = O is more electrophilic than is P = S, any change from S to O greatly increases the rate of hydrolysis. Water hydrolysis is favoured at an alkaline pH and strong alkali hydrolysis can be used to destroy OP toxicity.

The oxidation of P = S to P = O is another very common and important reaction that occurs in both abiotic and biotic conditions. The conversion of phosphorothioates to the homologous phosphates greatly enhances their toxicity to insects and mammals.

A typical reaction occurring with O-phosphorothioates is the thion-thiol isomerization under the influence of light and temperature. Thus, O-phosphorothioates are converted into S-phosphorothioates, which are in general more toxic than the parent compounds.

Solubility in water varies greatly from one compound to another. Most of OP esters are scarcely soluble in water but some of them are freely soluble and can be formulated in water-based medium.

It must be remembered that commercial formulations of OP pesticides may not contain only a single compound but rather be a mixture of OP's. Besides deliberately added substances, commercial products may also contain production impurities, sometimes in remarkable concentrations. The biological behaviour and toxicity to humans of OP associations may be different from those of the single compounds because metabolic interactions may result in synergic effects (see the example of malathion; further on pag. 66).

Mechanism of action

Organophosphorus pesticides exert their biological actions acting as inhibitors of enzymes. Esterases (or ester hydrolases) are the target enzymes responsible for OP toxicity to insects and mammalian species. Toxicity of a given dose of an OP compound to an animal organism primarily depends on the inhibiting power (affinity) of the OP against esterases and on the ability of that organism to activate or inactivate the OP.

Cholinesterase inhibition

Organophosphorus insecticides produce their acute toxic actions by inhibiting cholinesterase (ChE), the enzyme performing the hydrolytic cleavage of acetylcholine into choline and acetic acid. This mechanism of action is demonstrated by the fact that many of these compounds are inhibitors of ChE **in vitro**, and the inhibition of ChE can be measured **in vivo** after administration of OP insecticides. Additionally, atropine and other cholinergic blocking agents protect against acute OP poisoning and reversal of ChE inhibition which may be pharmacologically induced in intoxicated patients with oxime derivatives , results in alleviation of symptoms of poisoning.
Acetylcholine acts as a neurotransmitter of nerve action potentials of all preganglionic autonomic fibers, all post-ganglionic parasympathetic fibers and a few post-ganglionic sympathetic fibers. Moreover acetylcholine is the neurohumoral transmitter of the skeletal muscle motor end-plates and some interneuronal synapses in the central nervous system. The synaptic transmission of nerve potentials requires that acetylcholine is liberated in the intersynaptic space, linked by the postsynaptic receptor and destroyed within milliseconds, thus allowing the impulse transmission to end. This last task is normally performed by acetylcholinesterase (AchE), an enzyme located mainly in the nervous system and in the motor plates of skeletal muscle. When AchE is inhibited, post synaptic cholinergic transmission is not ended within the proper time and this results in a protracted cholinergic overstimulation.

There are two principal types of cholinesterases in the human organism:

I. **acetylcholinesterase** (other names: acetylcholine acetylhydrolase (EC 3.1.1.7.), specific cholinesterase, erythrocyte cholinesterase) and

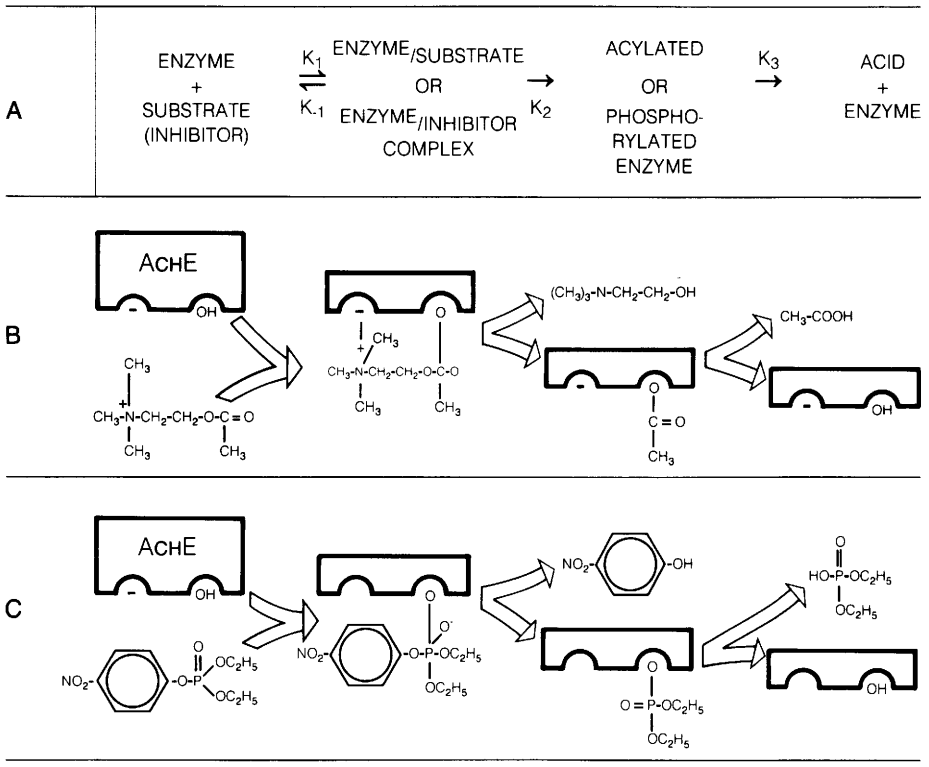
II **butyryl-cholinesterase** (PchE) (other names: acylcholine acylhydrolase (EC 3.1.1.8.), aspecific cholinesterase, pseudocholinesterase, plasma or serum cholinsterase).

They differ in location in tissues, substrate affinity and physiological function.
AchE hydrolyzes acetylcholine at a greater velocity than any other choline ester. Besides nervous tissue, AchE is also present in erythrocytes, where it is located on the cell membrane. The function of AchE in red blood cells is unknown.
PchE (or plasma cholinesterase) is a generic term covering a highly inhomogeneous group of enzymes, sometimes divided into butyryl and propionylcholinesterase according to substrate affinity. In the nervous tissue, PchE activity is present in glyal cells but not in neurons. Moreover PchE is present in plasma, liver and some other organs. Its physiological function is unknown. In human sera at least 4 isoenzymes have been identified by various techniques. In some subjects there is an absolute deficiency of plasma cholinesterase which is replaced by an atypical plasma esteratic enzyme. Atypical enzimes are seen in 3% of the population together with the normal PchE (heterozigous subjects). Very few subjects (homozigous; about 1/10.000) have a complete absence of normal PchE, which is entirely replaced by atypical variants. Atypical enzymes can be characterized by their inhibition pattern (for instance, resistance to dibucaine inhibition). These atypical enzymes are the result of genetic modifications in the locus that determines the formation of cholinesterase.

Practically all the pharmacological effects induced by OP compounds in the organism are due to the inhibition of AchE with the consequent accumulation of endogenous acetylcholine.
PchE is inhibited as well, but its inhibition at most sites produces no apparent functional derangement.

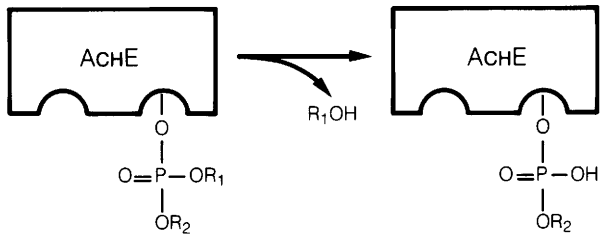
The mode of interaction of substrates and inhibitors with acetylcholinesterase is schematized in Figure 8, which shows the reaction of AchE with the physiological substrate, acetylcholine, and an OP insecticide, paraoxon. There are three important steps in the reaction: the first is complex formation and is governed by an affinity constant Ka ($K—1/K_1$). This is quite small for the OP insecticides as well as for the natural substrate acetylcholine; therefore formation of enzyme-substrate or enzyme-inhibitor complex is favoured. With acetylcholine K_2 and K_3 are both very fast so that the total reaction occurs rapidly and new active enzyme is regenerated. With OP esters K_2 is moderately fast, but K_3 is extremely slow, so the phosphorylated enzyme accumulates while the amount of reversible complex is minimal at any time.

Fig. 8 Reactions of acetylcholinesterase (AchE) with inhibitors or substrates.
A. Scheme of the whole reaction.
B. Hydrolysis of the physiological substrate acetylcholine.
C. Reaction with paraoxon.



The number of molecules hydrolyzed per minute by one molecule of enzyme has been estimated to be 10^7 to 10^8 times less for OP compounds than for acetylcholine. Therefore, the enzyme rapidly hydrolyzes acetylcholine, but is rather irreversibly inhibited by the OP's. The rate of recovery of free and active acetylcholinesterase following poisoning by OP compounds varies with different compounds, the great majority of OP's leading to long but reversible inhibition. Spontaneous reversal of enzyme inhibition occurs through the hydrolysis of the phosphorylated cholinesterase.
With some compounds a phenomenon known as «aging» of the phosphorylated enzyme occurs. Aging consists of a dealkylation of one of the groups linked to the phosphorous moiety of the complexes and leaves a hydroxy residue free on the phosphorylated enzyme (Figure 9). This reaction is important for the treatment of poisoned patients because

Fig. 9 Aging of phosphorylated acetylcholinesterase (AchE).



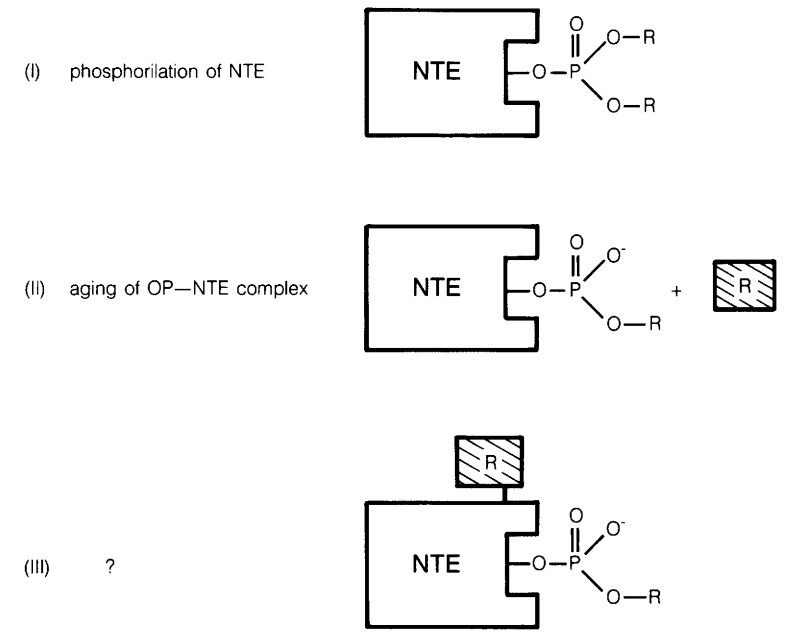
the capacity of the oxime derivatives to reverse cholinesterase inhibition **in vivo** depends on whether or not such reaction has already taken place. Since the «aged» phosphorylated enzyme cannot be reactivated by the oxymes, the effectiveness of treatment with these substances in antagonizing the poisoning is directly correlated with the earlyness of administration and inversely related to the rate of aging of the single OP compounds.

«Neuropathy Target Esterase» Inhibition

Delayed neurotoxic action of some OP pesticides is independent of cholinesterase inhibition but related to the phosphorylation of a specific esteratic enzyme in the nervous tissue,

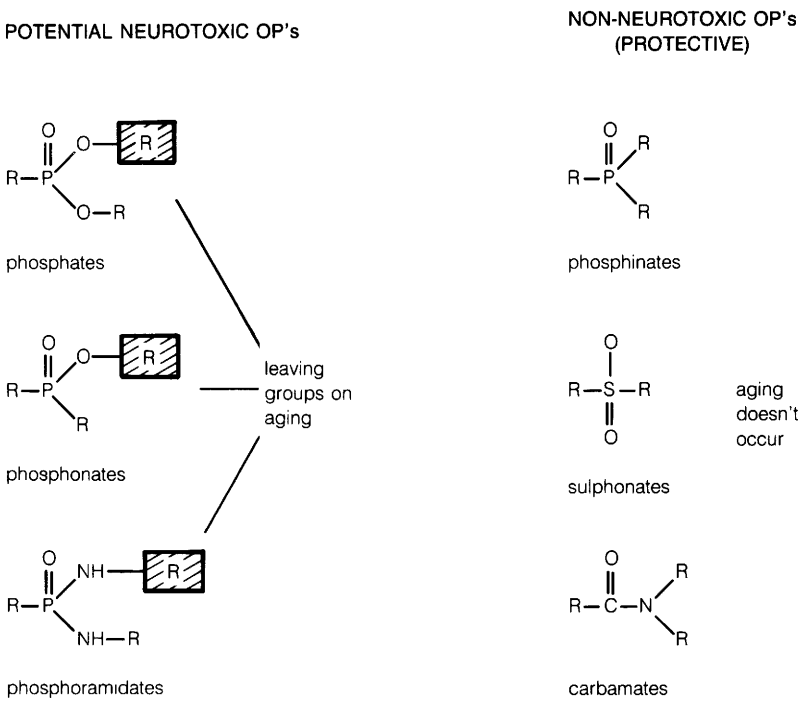
which has been denominated «neurotoxic esterase» or «neurophathy target esterase» (NTE) (1). The sequence of biochemical events at the receptorial site leading to the development of the delayed neuropathy has been in part elucidated (Figure 10). The initial biochemical

Fig. 10 Events triggering organophosphorus-induced delayed neuropathy.



reaction is represented by the phosphorylation of NTE. An essential second step is the transformation of the phosphorylated target to an «aged» form in which one group attached to the phosphorus has been cleaved and a negatively charged residue remains attached to the protein. The occurrence of this aging reaction, which is analogous to the aging of AchE-OP complexes, depends only on the chemistry of the OP and may only occur with phosphates, phosphonates and phosphoramidates. Compounds such as phosphinates and carbamates are not able to age (Figure 11) and if they link to NTE before

Fig. 11 Anticholinesterase agents may be neurotoxic or protective for delayed neuropathy according to whether they can age or not.



an axonopathic OP is administered, they block the receptor preventing the development of the neuropathy (2).
Identification of NTE and evidence for a direct involvement of this enzyme in the delayed neuropathy was achieved from animal experiments demonstrating the selective binding of labelled axonapathic OP's to an esteratic protein in the nervous tissue (3, 4). Since the nervous tissue contains a number of esteratic enzymes and none of them has an absolute specificity of substrate, the activity of NTE can be isolated and measured in the nervous tissue only after suppressing other irrelevant esteratic enzymes. This can be partially performed by administering an OP compound such as for instance paraoxon (POX), which is a potent inhibitor of AchE and many other esterases but does not cause delayed neuropathy. If one administers an adequate dose of paraoxon to an animal species sensitive to delayed neuropathy (usually the hen), AchE is strongly inhibited and the total esteratic activity of the nervous tissue decreases to less than 50%, but the animal is still sensitive to develop delayed neuropathy if dosed with an axonopathic compound. This indicates that NTE belongs to the fraction of POX-resistant esterases.
On the other hand, after administering an axonopathic OP (for instance, mipafox or DFP, diisopropylfluorophosphate), some esteratic activity is still detectable in the nervous tissue and this indicates that there are esteratic enzymes which are resistant to both paraoxon and mipafox or DFP (5).
NTE may be biochemically defined as the esteratic activity which remains after inhibition of nervous tissue esterases by non-axonopathic OP's and which is sensitive to inhibition of axonopathic compounds (6). Figure 12 shows the fractioning of esterase activity of the rat brain tissue which is obtained through the selective inhibition with a non-axonopathic OP (paraoxon) followed by an axonopathic one (mipafox). (7).

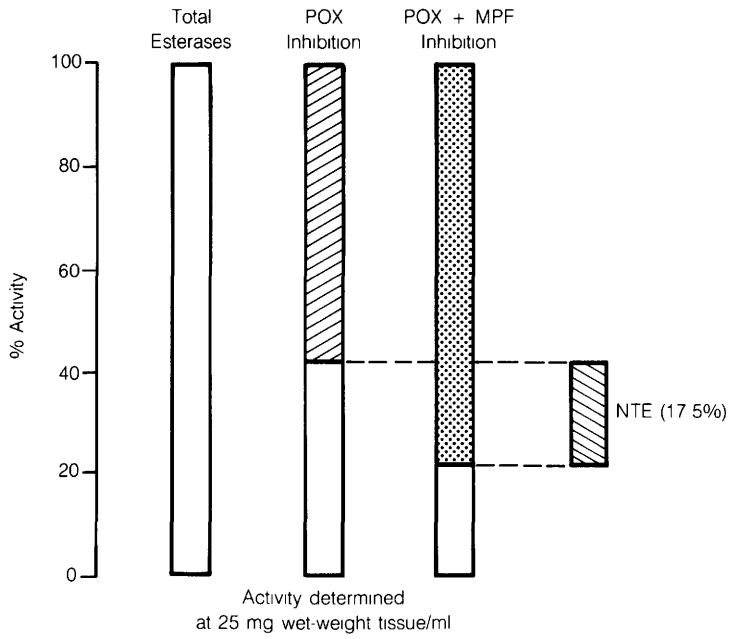


Fig. 12 Phenylvalerate-hydrolyzing esterase fractions of rat brain according to paraoxon (40 μM) or paraoxon plus mipafox (40 μM + 50 μM) inhibition

Different animal species are not all equally sensitive to develop delayed neuropathy from OP's. Within each species, susceptibility varies with age, young animals being almost totally resistant (1). The animal model of choice for the investigation of delayed neuropathy from OP's has been the hen. In order to cause delayed neuropathy in the hen, a minimum NTE inhibition of 70% **in vivo** has to be reached within 24 hours after OP administration. Whether a threshold exists in humans too and what its value is, is not known as yet, nor is it known what happens to the neurons as a consequence of the above reactions involving NTE. The esteratic activity itself of NTE may not be considered a biochemical function indispensable to the health of neurons as it may be suppressed without provoking neurophathy. Defective protein synthesis or impaired axonal flow have been conjectured as putative mechanisms of the delayed neuropathy, but no experimental evidence supports these hypotheses.
The physiological function of NTE, if any, is unknown. In man NTE is present in the nervous tissue, in liver, in lymphocytes, in platelets and in other tissues. (8).

Experimental studies in the hen have indicated that, after administration of axonopathic OP's, inhibition of NTE in blood lymphocytes correlates with NTE inhibition in central as well as peripheral nervous tissue. (9, 10). As will be further on detailed, this observation has lead to the attempt to develop biological indicators for monitoring the human risk of delayed neuropathy from OP's.

Effects of OP pesticides on humans

Acute Effects

The OP pesticides present a serious hazard of acute intoxication which varies considerably from compound to compound. The TD_{50} of OP's in mammals vary from mg to g/kg body weight (30). Some OP's are not active as such but need to be activated in the organism. Symptoms and signs of acute OP intoxication are given in Table I (36). When only local exposure occurs, signs and symptoms may be confined to the site of contact and spare the rest of the organism. The immediate cause of death in fatal OP poisoning is asphyxia resulting from respiratory failure.

Table 1. Signs and symptoms of anticholinesterase poisoning

Site of action	Signs and symptoms
	LOCAL EXPOSURE
Pupils	Pin-point miosis
Ciliary body	Frontal headache, eye pain, dimness of vision
Conjunctivae	Hyperaemia
Mucous membranes	Rhinorrhoea, hyperaemia
Bronchial tree	Tightness in chest with prolonged wheezing, bronco-constriction and increased secretion, cough
Sweat glands	Sweating at the site of contact
Striated muscle	Fasciculations at site of contact
	SYSTEMIC ABSORPTION
Bronchial tree	Tightnees in chest with prolonged wheezing, broncho-constriction, increased secretion, dyspnoea, slight pain in chest, cough
Gastrointestinal system	Anorexia, nausea, vomiting, abdominal cramps, epigastric and substernal tightness, eructation, diarrhoea, tenesmus, involuntary defecation
Sweat glands	Increased sweating
Salivary glands	Increased salivation
Lacrimal glands	Increased lacrimation
Pupils	Slight miosis (occasionally unequal), later marked
Ciliary body	Blurring of vision
Bladder	Frequent or involuntary micturition
Striated muscle	Fatigue, weakness, muscular twitching, fasciculations, cramps, generalized weakness including muscles of respiration with dyspnoea and cyanosis
Sympathetic ganglia	Pallor, occasional elevation of blood pressure
Central nervous system	Giddiness, tension, anxiety, jitteriness, restlessness, emotional lability, excessive dreaming, insomnia, nightmares, headache, tremor, apathy, withdrawal and depression, bursts of slow waves of elevated voltage in EEG especially on hyperventilation, confusion, slurred speech, ataxia, convulsions, coma
Circulatory system	Bradycardia, decreased cardiac output, cardiac arrest. paralysis of vasomotor centre

(from 36, modified)

The interval between exposure and the onset of symptoms may be as short as a few minutes or last some hours. The duration of symptoms is generally from 1 to 5 days and in non-fatal cases symptomatology may subside without leaving permanent sequelae. Onset and duration of the acute phase as well as severity of the poisoning may vary with the route of exposure and the absorbed dose, and differ markedly for different compounds according to their rate of biotransformation and affinity for cholinesterase.

Confirmation of the nature of the poisoning is usually achieved by the finding of a marked inhibition of erythrocyte or plasma cholinesterase.

Chronic or delayed effects

The great majority of the OP compounds in use are rapidly metabolized and excreted, and subacute or chronic poisoning due to accumulation of the compounds in the organism does not occur. However, because several OP compounds cause slowly reversible inhibition of cholinesterase, accumulation of this effect can occur. Thus, signs and symptoms resembling those occurring after a single high dose may be produced by repeated small doses absorbed on chronic exposure.

Certain OP pesticides can also produce a delayed neuropathy which is totally independent of their cholinesterase-inhibiting power. This neuropathy is the same as that caused by other organophosphorus esters such as triorthocresylphosphate (TOCP) which are deprived of anticholinesterase activity and not used as pesticides (11). The pathological and clinical picture of delayed neuropathy from OP pesticides is well known in animals (12). In man only a limited number of cases have been reported, but numerous epidemics of peripheral neuropathy involving thousands of subjects have been described for TOCP (11, 13).

Delayed neuropathy from OP's may occur both as a sequela of acute intoxication or after prolonged exposure. Clinical onset of the neuropathy occurs 1 to 3 weeks after exposure. The main symptoms, cramping muscle pain and progressive weakness, begin in the legs with ascending progression and may eventually involve the arms. Subjective sensory loss is typically mild or absent. On examination, depression of tendon reflexes and flaccid weakness of the distal limb muscle are the outstanding signs. Mild to moderate pyramidal signs may be present as well. The pathological picture is typical of a distal axonopathy with proximally progressive nerve degeneration. Axonal lesions primarily involve the large-diameter fibers of the long nerves of the limbs and the long spinal cord tracts, particularly in the dorsal columns. Electromyographic examination of these patients reveals a pattern of partial denervation of the affected muscles and electroneurography shows an appreciable delay in terminal motor latencies while maximal motor conduction velocity is normal or very slightly decreased in most cases. Recovery from the neuropathy is very slow and may be incomplete as indicated by the observation of functional impairments still lasting years after the onset of the neuropathy (14).

Numerous OP pesticides are able to induce delayed neuropathy in experimental animals and the structure-activity relationship has been in part identified (2). So far human cases have been reported after exposure to mipafox (15), leptophos (16), trichlorphon (17), trichloronate (18), and methamidophos (19).

Investigations on workers exposed to OP pesticides during their manufacture have also shown transient modifications of some electromyographic and electroneurographic parameters (20, 21). These findings however have not been confirmed in extended studies and their significance has been questioned.

Persistent central nervous system alterations have been sometimes described in patients recovering from acute OP intoxication as well as in subjects chronically exposed to OP's (23-26). Impaired vigilance and reduced concentration, slowing of psychomotor speed and information processing, memory deficit, linguistic disturbance, depression, anxiety and irritability have been the most frequently reported behavioral changes. Although the studies available on human behavioral changes by OP pesticides are incomplete and sometimes inconsistent, they indicate an area warranting more systematic and definitive investigation.

In surveys on workers exposed to OP's alone or in combination with other pesticides, abnormalities of blood chemistry parameters or serum enzymes other than PchE have been occasionally described. However there is no rigorous epidemiological evidence that these effects have to be attributed to OP exposure on a cause-effect basis. In animals, OP pesticides show a low-to-moderate liver toxicity and toxic liver injuries are not a common finding in human acute intoxications. Therefore liver deserves much less emphasys as a target organ of OP toxicity, than is usually laid on it.

Recent investigations have shown that several OP pesticides, including parathion, diazinon, chlorfenviphos and azinphosmethyl, can induce porphyrinogenic effects in experimental conditions (27). Human cases of porphyria have been reported after exposure to diazinon and dicrotophos (28).

OP pesticides have always been considered to be only inhibitors of enzymes. However a study on exposure to malathion during its production has documented a shortening of antipyrine half-life in the workers (29). This would suggest that OP compounds may also be inducers of liver microsomal enzymes.

Metabolism

Absorption and distribution

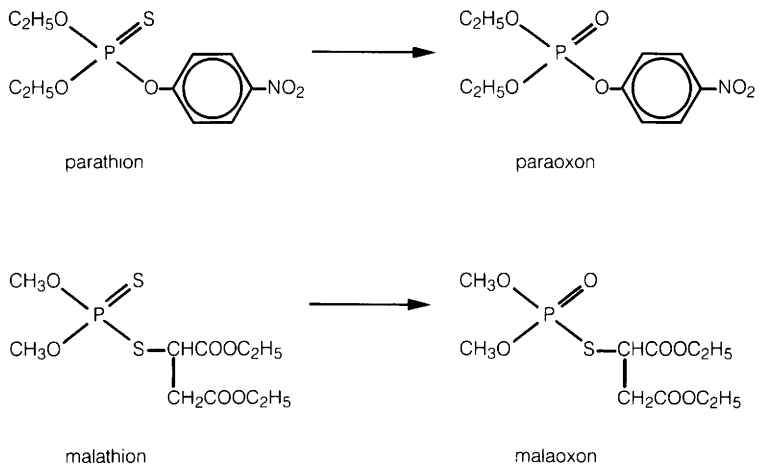
OP pesticides may enter the organism through inhalation, ingestion and percutaneous absorption. The relative importance of these routes of entry varies according to the physicochemical properties of the OP, the exposure condition, and the nature of the formulation of the pesticides.
Inhalation is mainly important during the production of pesticides in chemical plants, their formulation and their diffusion by aerial spray.
Percutaneous absorption may occur especially during formulation when the pesticides are improperly handled, or during application in agriculture or in public health. Ability of the different OP's to penetrate the skin varies from one compound to another but is generally enough to produce acute intoxication if the operators are not adequately protected. Specific studies on skin penetration of the different OP's in animals and man are available and the subject has been recently reviewed (30).
Absorption of OP's through the digestive tract has been demonstrated by reports of acute intoxications following oral ingestion in man. Digestive tract absorption due to occupational exposure may occur when personal hygienic practices are poor or workers consume food in contaminated areas. This route of absorption is also relevant to the possibility of OP intake by the population as a consequence of consumption of vegetables or fruits contaminated with OP residues.
Investigations with different OP's have shown that the bioavailability of their active forms in the organism is less after digestive absorption than after other routes of exposure. This is explained by the «first pass» phenomenon, that is the greater inactivation occurring in the liver as compared with other tissues when the compounds pass through this organ before accessing distribution via the general circulation.
After absorption, OP pesticides and their metabolites distribute quickly in all tissues. The maximum concentrations are usually found in liver and kidney; lipophilic compounds may reach remarkable concentrations in nervous tissue or other lipid-enriched tissues.
Plasma half life of OP pesticides after single administrations ranges from minutes to few hours, depending on the compound and the route of administration. For some compounds there is a marked interindividual and inter-species variation in biokinetics; for this reason, the extrapolation of animal data to man must be cautious.

Biotransformation

The relationship between enzymatic biotransformation and toxicity of OP pesticides is extremely complex.
Toxicity depends upon the net availability of active compound to inhibit the specific enzymatic targets at critical sites in nerve tissue, and this in turn depends upon the dynamic relationships between activation and inactivation reactions.

Activation. All thiono OP pesticides, that is those containing a P = S bond, are not active inhibitors of AchE, but require activation by oxidation of the P = S to the P = O group, thus producing the corresponding oxygen analogues (Figure 13).

Fig. 13 Activation of phosphorothioates and phosphorodithioates



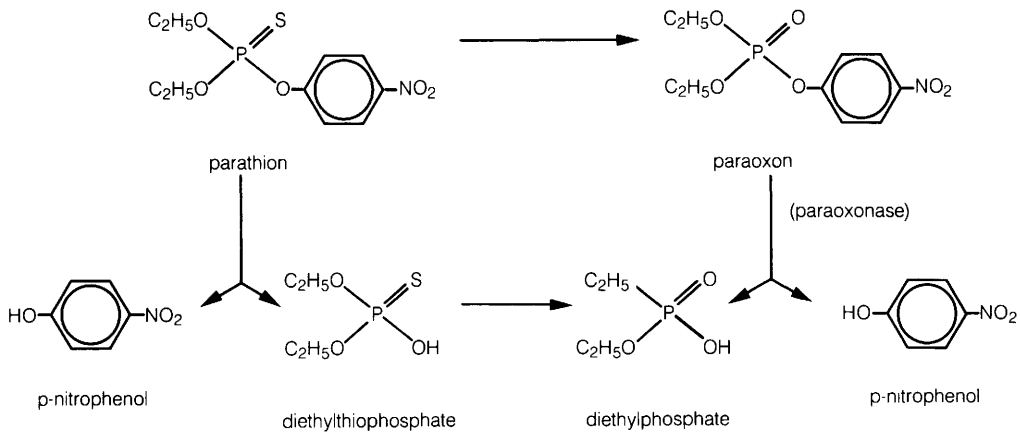
The enzyme system in liver that catalyzes this reaction belongs to the group of NADPH-dependent mixed-function oxidases of the microsomes. Although the liver has the greatest capacity to perform this reaction, this bioactivation may also occur in other tissues, including lung and brain. Activation of thiono OP compounds by extrahepatic tissues, even if only in minimal amounts, may be of importance if these tissues are critical target organs for AchE inhibition.

Inactivation. Detoxification of OP pesticides in the organism can occur either by biochemical modifications of their structure or by their linkage to binding sites of the organism having no toxicological significance.
The main reactions yelding products deprived of anti-ChE activity are:

1. breakage of the P-X bond by esterases;
2. oxidation of the alkyl side chain (R_1 or R_2) so that the R-O-P bond becomes unstable and breaks;
3. transfer of the alkyl group to glutathione by the action of glutathion S-transferase;
4. breakage of ester bonds by carboxylesterases.

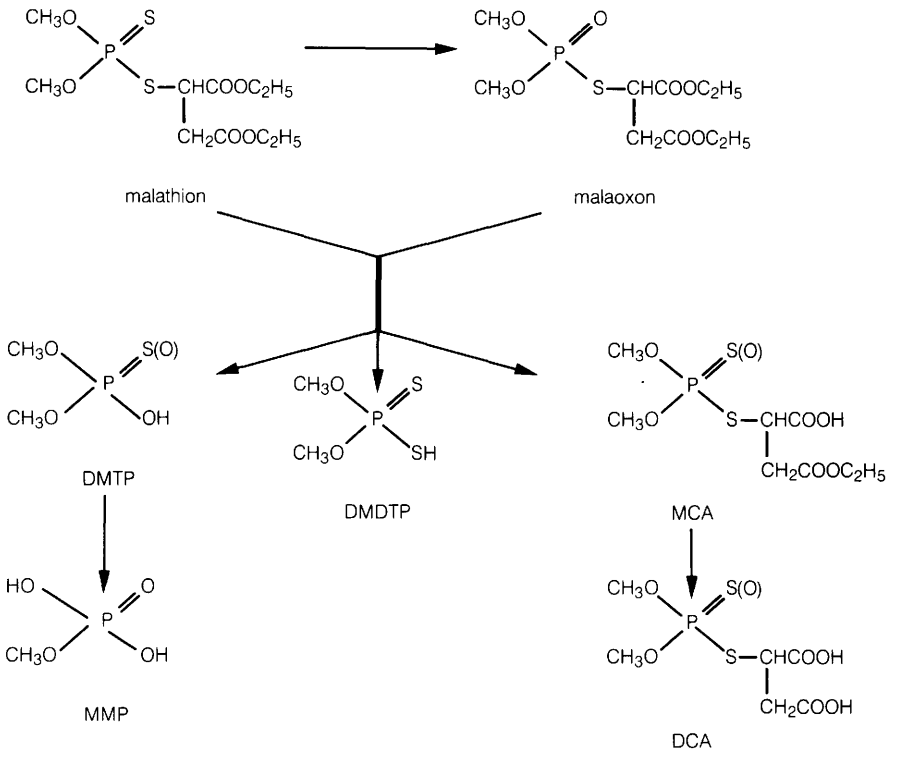
The first reaction may be exemplified with the biotransformation of parathion (Figure 14).

Fig. 14 Metabolic pathways of parathion.



Parathion after conversion to its oxygen analogue, paraoxon, is hydrolized with formation of free p-nitrophenol. Hydrolysis of paraoxon is performed by an enzyme, paraoxonase, which is present in serum, liver and other tissues of animals and the man. The aryl-phosphorus bond can also be cleaved without prior oxidation of parathion to paraoxon. This reaction is catalyzed by a NADPH-dependent microsomal enzyme which appears to be distict from the enzyme that converts parathion to paraoxon. The rate at which this oxidative cleavage or the combined oxidation-hydrolysis occurs, determines the amount of paraoxon available in tissues for the inhibition of AchE.
Dealkylation reactions, either oxidative or through the action of glutathion alkyltransferase, occur to only a very minimal extent with parathion, but are very important for several other compounds.
Oxidative dealkylation is performed by the microsomal system of the liver. For some OP's, such as for instance chlorfenviphos, inactivation by this reaction is substantial (31).
Since there is a marked interspecies variation of the relative rate of this system, acute toxicity of chlorfenviphos in different animal species is strictly dependent on the efficiency of this detoxificating pathway.
Other compounds, including methylparathion and azinphosmethyl, are preferentially monodealkylated by glutathion alkyltransferase (32), the activity of which is influenced by the availability of liver glutathion.
Hydrolysis of ester or amidic bonds located in the lateral chains of the molecule is an important inactivation reaction for compounds such as malathion and dimethoate (Figure 15). This reaction is carried out by tissue or plasma carboxylesterases (or carboxylamidases) and the products of hydrolysis are not inhibitors of cholinesterase. Since carboxylesterases are serine-esterases which can be inhibited by other OP compounds, combined exposure to different OP pesticides may potentiate the acute toxicity of malathion by blocking its

Fig. 15 Metabolic pathways of malathion.



MCA : malathion(malaoxon)—α—monocarboxylic acid
DCA : malathion(malaoxon)—dicarboxylic acid
DMDTP : dimethyldithiophosphate (or dimethylphosphorodithioate)
DMTP : dimethylthiophosphate (or dimethylphosphorothioate)
MMP : monomethylphosphate

detoxification (33, 34). Such a phenomenon is believed to have occurred in Pakistan where in 1978 an acute epidemics of malathion poisoning affected thousands of people working for a malaria control program. The epidemics broke out when a brand of malathion contaminated with iso-malathion and other minor impurities was used. As malathion per se is one of the safest OP pesticides for man, the cause of the mass poisoining was attributed to the metabolic potentiation of its toxicity through the inhibition of carboxylesterases induced by the impurities (35).

All the reactions discussed above may be considered true inactivation mechanisms, in that the active OP compounds are biotransformed with formation of less active or totally inactive metabolic products. There is another major mechanism of detoxification which does not operate through metabolic conversion of the OP but rather by sequestering the active forms of the OP's at binding sites of the liver and other tissues. These binding sites, which are very likely represented by non-specific tissue esterases, may exert a great buffer capacity, thus sparing the critical AchE of nerve tissue from inhibition.

Elimination

Elimination of OP's and their metabolites occurs mainly via the urine and the faeces, being fecal elimination mediated through hepatic transport into the bile.
Urinary and fecal excretion is usually rapid. With most compounds 80-90% of the dose is eliminated within 48 hours, although small amounts of OP's and their metabolites can be recovered in urine for a few days.
A very little proportion of the OP's and their active forms (the oxons) is eliminated unchanged in the urine.
The by far largest part of the compounds present in excreta is represented by alkylphosphates and the residues resulting from the hydrolysis of the specific groups attached to the posphorus. Rate of elimination and relative proportion of each metabolite varies over time and with different compounds.
More detailed information on the metabolism and the specific products of elimination of some OP's in man will be found in Table 6.

Biological indicators

In recent years, while advances in analytical chemistry have offered improved possibilities of measuring pesticides and their metabolites in the organism, comparatively less progress has been made in the acquisition of early and reversible indicators of their toxicity in humans. On the other hand, the wider availability of analytical possibilities itself has seldom resulted in concrete improvements of routine monitoring because problems of cost and the multiplicity of products handled by agricultural or public health workers hamper its practical application.

Human exposure to pesticides has different characteristics according to whether it occurs during their industrial production or their use (Table 2) (35). The formulation of commercial

Table 2. Comparison between exposure characteristics during production and use of pesticides.

	Exposure on production	Exposure on use
Duration of exposure	Continuous and prolonged	Variable and intermittent
Degree of exposure	Quite constant	Extremely variable
Type of exposure	To one or few compounds	To numerous compounds either in sequence or concomitantly from use of mixtures
Skin absorption	Easy to control	Variable according to work procedures
Ambient monitoring	Useful	Seldom informative
Biological monitoring	Complemental to ambient monitoring	Very useful when available

(from 35, modified)

products by mixing active ingredients with other coformulants, has some similarities with use as far as exposure characteristics are concerned. In fact these activities are typically performed by small industries which manufacture many different products in subsequent campaigns. Thus, the workers are exposed to several pesticides and to each of them for a short time. In public health and agriculture, the usage of a variety of compounds is generally the rule, although in some specific applications (for example, cotton defoliation or malaria control programs) a single product may be used.
Usage of biological indicators of exposure is particularly necessary in pesticide utilization where the conventional techniques of ambient monitoring are scarcely applicable. Moreover, the possibility of percutaneous absorption makes the use of biological indicators very important to assess the level of exposure in every circumstances.

Indicators of internal dose

For the majority of OP pesticides, the blood or urine concentration of the substance itself or its metabolites represent an indicator of exposure. However, in order to use plasma or urine concentrations as indicators of internal dose, metabolism and pharmacokinetics of each compound should be known with reference to studies in humans, since extrapolation from animal species to man can be only tentative and qualitative.

Although other routes of elimination are active, the determination of metabolites in the urine is the most practical mean for the estimation of the internal dose of the OP pesticides. Taking into account the general features of the OP biotransformation, two complemental approaches may be used: the determination of metabolites deriving from the alkylphospate moiety of the molecule or the measurement of the lateral-chain residues which are generated by the hydrolysis of the P-X bond.
Both these methods have been attempted, but a variable success has been achieved for different compounds, according also to the analytical methods used.

The measurement of p-nitrophenol in urine to assess the internal dose of parathion has been among the oldest tests developed. Favoured by the availability of colorimetric analytical methods, this test has been widely experimented and has proved to be successful in evaluating exposure to parathion (30). Parathion-methyl and EPN are other pesticides which yeld p-nitrophenol in urine (37). Exposure to fenitrothion may be surveyed by measuring p-nitrocresol in urine (38).
Less experience has been obtained with OP's bearing other chemical structures in the lateral chain. The measurement of the product of hydrolysis of the P-X bond seems to be analitically rather easy when the metabolic residues to be detected are phenolic or

chlorophenolic derivatives, as in the case of chlorfenviphos or fenchlorphos. When the metabolites originating from the cleavage of the P-X bond are complex heterocyclic structures, as occurs f.i. with azinphos-methyl, or non-specific chemicals as occurs with malathion, this approach cannot be successfully pursued.
The alternative consists of measuring alkylphosphates in urine. The rationale for the use of this method resides in the fact that metabolism of most OP's yelds alkylphosphates or alkyl(di)thiophosphates as terminal products. Since these metabolites are common to several different OP's, this method is not compound-specific and is only usable to assess exposure to all the parent compounds which may generate these derivatives. This fact, if advantageous when multiple exposure has to be monitored, represents a limit for the toxicological interpretation of results, because each OP pesticide has its own metabolic rate and toxicity level. The various alkylphosphates detectable in urine and the main parent compound they can originate from, are listed in Table 3.

Table 3. Organic phosphates detectable in urine as metabolites of OP pesticides

Metabolite	Principal parent compounds
Monomethylphosphate (MMP)	Malathion
Dimethylphosphate (DMP)	Dichlorvos, trichlorfon, mevinphos, malaoxon, dimethoate, fenchlorphos
Diethylphosphate (DEP)	Tetraethylpyrophosphate, paraoxon, demeton-oxon, diazinon-oxon, dichlofenthion
Dimethylthiophosphate (DMTP)	Fenitrothion, fenchlorphos, malathion, dimethoate
Diethylthiophosphate (DEPT)	Diazion, demeton, parathion, fenchlorphos
Dimethyldithiophosphate (DMDTP)	Malathion, dimethoate, azinphos-methyl
Diethyldithiophosphate (DEDTP)	Disulfoton, phorate
Phenylphosphonic acid	Leptophos, EPN

The measurement of alkylphosphates in urine requires analytical methods rather sophisticate, based on derivatization of the compounds and separation and detection by GLC (39, 40). So far the measurement of alkylphosphates has been performed in few studies, mostly addressed to research purposes.

With some OP pesticides, the assessment of the internal dose may be performed by determining urinary metabolites which result from a partial degradation of the molecule at a site other than the phosphorus bonds. For instance, the major urinary metabolites of malathion are the mono- and dicarboxylic phosphorus-containing acids deriving from the hydrolysis of the diethylsuccinic ester in the lateral chain (35).

Knowledge about the relationship between exposure and the internal dose of OP's as determined by urinary metabolites measurement, is very limited. With the exception of parathion and few other compounds, no data are available in humans. Some information has been obtained from animal studies, but the routes of exposure used in these experiments are hardly representative of human exposure conditions, especially as far as occupational exposure is concerned. To date this is the major drawback against the use of these bioindicators.
Some studies have investigated the relationship between level of urinary OP metabolites and serum or red blood cell AchE activity in humans. Detectable amounts of urinary metabolites without evidence of AchE depression have been found in some cases (30). This indicates that biological assessment of exposure through urinary metabolite determination may be a very sensitive method, capable of revealing exposure in a range of doses which are insufficient to bring about a toxic response. This very important feature accounts for the interest in this methodology despite its practical and analytical difficulties.

OP metabolites are usually excreted in the urine within a short time and the peak of emission occurs few hours after the beginning of exposure.
Therefore in occupational exposure samples collected soon after the end of the work are suitable for metabolite determination when 24 hour urine collection is impractical. When using spot specimens, creatinine or specific gravity should be determined in order to select and discard those samples which are too diluted or concentrated.
Trace amounts of alkylphosphates have been found in some studies in the urine of subjects considered non-exposed to OP's (39,41). However, since endogenous sources of

alkylphosphates others than OP esters have never been reported, the presence of alkylphosphates in urine should be highly specific for OP exposure. According to the National Human Monitoring Program of the U.S. Environmental Protection Agency, the frequency of occurrence of dialkylphosphate residues in the urine of the general population is rather low (Table 4) (42). Until more definite knowledge on this matter is acquired, comparison with reference groups or with individual pre-exposure values is recommended when surveying workers with occupational exposure.

Table 4. Occurrence of dialkyl phosphate residues in human urine

Dialkyl phosphate residue[a,b]	Frequency of detection, %
Dimethyl phosphate (DMP)	12
Diethyl phosphate (DEP)	7
Dimethyl phosphorothionate (DMTP)	6
Diethyl phosphorothionate (DETP)	6
Dimethyl phosphorodithioate (DMDTP)	1
Diethyl phosphorodithioate (DEDTP)	1

a) Based on analysis of 5676 specimens collected in U.S.A. on 1976-1980.
b) Limits of detection: 20 ppm.

(from 42, modified)

Indicators of effect

The receptors for the toxic action of OP pesticides reside in the nervous tissue and are not accessible for being measured with non-invasive methods.
Therefore, for the purpose of human biological monitoring, other indicators which are related to the receptors in the nervous tissue and are easily obtainable, must be used.

1) Red blood cell and serum cholinesterases.

Cholinesterase inhibition is the most prominent pharmacodynamic effect of exposure to OP pesticides.
It must be noted that cholinesterase inhibition is relevant only to acute toxicity of OP's and thus the assessment of cholinesterase inhibition is not by itself predictive of chronic or delayed effects.
The determination of acetylcholinesterase activity in red blood cells (AchE) or pseudocholinesterase activity in serum or plasma (PchE) have been the most reliable and widely used indicators of exposure.
A large and consistent body of evidence indicates that depression of red blood cell AchE activity as well as serum PchE activity is highly correlated with intensity and duration of single OP exposures. Red blood cell AchE, being the same molecular target as that responsible for acute OP toxicity in the nervous system, is an indicator more specific than PchE.
Some OP compounds such as for example malathion,diazinon and dichlorvos, are earlier inhibitors of PchE than AchE (30). With these substances serum PchE determination might be a more sensitive indicator of exposure than AchE; however PchE inhibition may not be associated with symptoms or signs of toxicity.
After a single OP exposure, serum PchE activity recoveries more quickly than AchE activity in red blood cells. After a severe intoxication, the reduction of enzyme lasts up to 30 days in plasma and up to 100 days in erythrocytes, corresponding to the time of liver PchE resynthesis and red cell replacement, respectively.

Interindividual coefficients of variation in cholinesterase activity of the general population have been determined in the region of 15-25% for the PchE and 10-18% for erythrocyte AchE (43-47). Corresponding figures for intraindividual variation are 6% and 3-7% respectively.
Erythrocyte AchE shows no difference in activity between the sexes when the sex-related difference in red-cell packed volume is taken into consideration (45-47). There is no significant variation with age, with the exception of the less than 6 month old infants who have values lower than adults. (47-48).
Plasma Pche shows normal values 10-15% greater in males than in females. Significant correlations have been found between PchE activity and body mass or serum cholesterol level (49, 50) and a multifactorial equation has been proposed to predict individual PchE values in healthy subjects based on physiological individual parameters (50).

No correlation has been observed between PchE activity and age. (51).
Repeated seasonal and circadian measurements of AchE and PchE in healthy subjects have not shown any periodical intra-individual variation. (47, 51).

A reasonable correlation exists between red cell and plasma AchE activity and the clinical signs of the acute intoxication.
The correlation tends to be better as the rate of inhibition is faster. When inhibition occurs slowly and repeatedly, as it happens on chronic or repeated exposure, the correlation with illness may be low or totally inexistent. For instance, after continuous exposure, clinical signs of intoxication may appear only at inhibition of 85-90% of the pre-exposure AchE level as opposed to the 60-70% inhibition level usually observed after a single exposure.
Table 5 summarizes the correlation existing between severity and prognosis of acute OP intoxication and the levels of AchE inhibition determined in peripheral blood.

Table 5. Severity and prognosis of acute OP intoxication at different levels of AchE inhibition

% AchE inhibition	Level of poisoning	Clinical symptoms	Prognosis
50-60	Mild	Weakness, headache, dizzines, nausea, salivation, lacrimation, miosis, moderate bronchial spasm	Convalescence in 1-3 days
60-90	Moderate	Abrupt weakness, visual disturbances, excess salivation, sweating, vomiting, diarrhoea, bradycardia, hypertonia, tremor of hands and head, disturbed gait, miosis, pain in the chest, cyanosis of the mucous membranes	Convalescence in 1-2 weeks
90-100	Severe	Abrupt tremor, generalized convulsions, psychic disturbance, intensive cyanosis, oedema of the lung, coma	Death from respiratory or cardiac failure

Quantitative relationships between exposure or absorbed dose and the effect of some OP's on cholinesterase in human subjects are given in Table 6. Studies with volunteers mostly cover oral administration and provided gastrointestinal absorption is complete and rapid, relationships can be calculated. Exposure to pesticides in manufacturing and application environments, however, involves respiratory and percutaneous absorption, mainly. The dose absorbed then should be estimated from air concentration and duration of exposure, taking also into account the opportunity for skin absorption. However, such estimates are rarely available and little is known on the influence of the route of exposure on metabolism of each pesticide.

The validity of testing cholinesterase activity in monitoring human OP exposure is limited because of the natural variation of blood cholinesterase activity in healthy people and the occurrence of false positive or negative results. Physiological variation as well as specificity are more favourable in AchE than in PchE use. The sensitivity of the test can be increased by adopting individual pre-exposure values as a reference.
Low values of PchE not related to OP exposure may be found in liver diseases (hepatitis, icterus, cirrhosis), uraemia, cancer, hearth failure and allergic reactions. In females, lower values are also measured during menstruation and pregnancy.
Increased values of PchE are present in hypertyroidism and other conditions of high metabolic rate.
With AchE, abnormally low values not related to OP exposure have been observed in subjects affected with leukemias or other neoplasmas. Increased values may be found in polycythemia and in thalassemia or other congenital blood dyscrasias. (52).

Sampling of blood depends on the purpose of the test and on the analytical procedure to be used. Venipuncture, although requiring trained personnel, should be preferred even when small samples suffice for determination of enzyme activity, because capillary blood from a finger or ear-lobe can be unproportionally contaminated with the chemical residing on the skin in occupationally exposed subjects. Every time it is possible, three sequential samples should be taken before exposure in order to establish a normal baseline for each worker.

Many analytical methods are available for the determinations of AchE and PchE. Most of them yeld comparable results, but accuracy and precision vary from one method to another.
Some methods have a sensitivity scale different from others, so that a given percentage of inhibition of a method may not be coincident in term of absolute activity with the same percentage of inhibition measured with a different technique.

Table 6. Available information in man on the relationship between exposure or internal dose of OP compounds and the effects on cholinesterases

Compound	Urinary metabolites detected in man (a)	Time course and/or recovery of urinary elimination	Exposure-dose and dose-effect relationship	No-effect level	Maximum acceptaple daily intake (mg/kg body weight) and year established (b)
Azinphos-methyl	DMP, DMTP (83)	i.v.-1,5% after 12 hrs		20 mg/man/day-oral (57, 58, 59)	0.0025 (1974)
Chlorfen-vinphos	Desethylchlorvinphos (DEC) (60)	oral-4,7% DEC at 2,5 mg/man/day; 23.8% DEC after 12.5 mg/day (60)	120 μg DEC/24 hrs urine excretion after 2.5 mg/man/day for 53 days (60)		0.002 (1972)
Chlorpyrifos	DEP, DETP, 3,5,6-trichloro-2-pyridinol (61,62)			0.03 mg/kg/day-oral (63)	0.01 (1983)
Demeton			40% PchE and 16% AchE depression after 0,1 mg/kg/day for 25 days (64)	0.05 mg/kg/day-oral (64)	No adi (1983)
Diazinon			60% PchE depression and unchanged AchE after 0.05 mg/kg/day for 28 days (65)	0.02 mg/kg/day (65)	0.002 (1976)
Dichlofenthion	2,4-dichlorophenol (66)				
Dichlorvos	DMP, dichloroethanol (67)		29% PchE depression and unchanged AchE after 2 mg/man/day for 28 days (68,69)	Thereshold for inhibition of PchE without AchE inhibition: 0.15 mg/m^3 for 16 hrs 0.4 mg/m^3 for 5 hrs (30)	
Dimethoate		70-100% excreted within 24 hrs (71)		0.2 mg/kg/day (70)	0.002 (1985)
Fenitrothion	3-methyl-4-nitrophenol (38) DMTP (82)	Peak excretion after 12 hrs; total amount excreted within 24 hrs (38)	5 mg/hr excreted after 10 mg oral dose (38)	0.33 mg/kg/day (38)	0.003 (1983)
Malathion	Malathion monocarboxylic acid (MCA), malathion dicarboxylic acid (MDA), DMP, DMTP, DMDTP (30,35)		No decrease in ChE at 3.6 mg/l MCA in urine (35,72); 25% PchE decrease after oral 24 mg/day for 56 days (73)	16 mg/day (73)	0.02 (1978)
Methidathion	In rats DMP and DMTP (74)			0.11 mg/kg/day (75)	0.005 (1976)
Mevinphos	DMP (76)	Excretion completed within 48 hrs (76)	25% AchE decrease after oral 2.5 mg/day for 27 days (77) 0.4 mg/l DMP in a case of moderate poisoning (76)	1 mg/day (77)	0.0015 (1973)
Parathion	p-nitrophenol, DEP, DETP, (37,78)	86% p-nitrophenol and DETP excreted within 8 hrs; DEP persists in urine for 48 hrs (37)	1,5 mg/l paranitrophenol in urine at the end of the work day corresponds to a 5 mg/man/day intake (30); 89% AchE and 63% PchE reduction after oral 7.2 mg/man/day (79)	0.07 mg/kg/day (79)	0.005 (1979)
Parathion-methyl	p-nitrophenol, DMP (37)	60% paranitrophenol excreted within 4 hrs; DEP excretion more protracted (37)	37% AchE reduction after oral 30 mg/day (80)	22 mg/day (80)	0.001 (1979)
Trichlorfon	Dichlorvos, dichloroethanol (81)			1-10 mg/kg/day (30)	0.01 (1979)

(a) = for abbreviations see Table 3
(b) = from the «Report of the Joint FAO/WHO Meetings on Pesticide Residues in Food», yearly published by WHO.

Calibration and comparability among different methods should be established in the same laboratory and among different settings. At a 1984 WHO meeting it was established that a modified version of the Ellman spectrophotometric method should serve as a reference for the multinational epidemiological study on neurotoxic effects of OP exposure which is being conducted in Europe under the coordination of the Office for Europe of WHO and the sponsorship of United Nations Development Programme (53). Recently paper-test methods have also been developed for screening purposes and field use in agriculture. These methods, though only semiquantitative, have low cost and ease of application.

Table 7 shows the values of AchE fitting the guidelines for biological monitoring established at the Workshop on Epidemiological Toxicology of Pesticide Exposure held in Amsterdam in 1971 (54).

Table 7. Values of AchE inhibition according to the criteria for biological monitoring established at the Workshop on Epidemiological Toxicology of Pesticide Exposure (Amsterdam, 1971)

Level	Significance	AchE inhibition (a)	Measures required
First level (no effect)	Values at which no physiological or biochemical effects are expected. Values usually found in normal population without exposure	—	No action needed
Second level (surveillance)	Values indicative of or compatible with minor and reversible effects	0-30 (1) 0-50 (2)	Medical surveillance needed. Working conditions to be examined to avoid exceeding such a level
Third level (effect)	Values indicative of or compatible with minor damages (initial symptoms, mild alterations of sensitive clinical indexes)	30-60 (1) 50-70 (2)	Temporary removal from exposure and analysis of working conditions needed

(a) By definition, any «effect test» cannot evaluate the first level.
(1) Based on individual pre-exposure baseline.
(2) Based on normal reference values.

2) Lymphocyte and platelet NTE.

The onset of delayed neurotoxic effects induced by some OP compounds has been shown to be preceded by inhibition of «Neuropathy Target Esterase» (NTE) in the nervous tissue immediately after exposure. Since NTE is present in circulating lymphocytes and platelets, the measurement of peripheral NTE might be informative of the events occurring in the nervous tissue of subjects exposed to OP esters.

Experimental data have indicated that after dosing animals with axonopatic OP's NTE inhibition measured in peripheral lymphocytes parallels inhibition in the central and peripheral nervous tissue (Figure 16) (9). In the first few hours after administration, the levels of NTE inhibition in blood and nervous tissue are almost coincident, while in the following days the rate of recovery of NTE activity is slightly more rapid in lymphocytes than in the peripheral nerves, due to different rates of NTE re-synthesis (Table 8) (55).

The existence of such a correlation has prompted the experimental testing of this method in subjects exposed to axonopathic OP's. Lotti and coworkers, monitoring cotton-defoliant sprayers, have found a consistent inhibition of activity in blood lymphocytes in the absence of any detectable sign of illness (56). The NTE activity decrease occurred during and after exposure and was preceded in some subjects by a transient increase in NTE activity soon after the beginning of exposure (Figure 17). The meaning of this diphasic phenomenon, which sometimes has also been observed in experimental animals, is still unknown.
This pilot application suggests that this method of monitoring may be promising to assess the risk of delayed neurotoxic effect. So far this test is still at a developmental stage and definite criteria of interpretation of the results in man are not yet available. Moreover the test presents inherent complexities, including the analytical method required for NTE assay in blood lymphocytes. Since a fair correlation has been observed between NTE activity in human lymphocytes and platelets (Figure 18), substituting platelets for lymphocytes may facilitate the application of the method (7). The reference values for NTE activity determined in two independent studies are shown in Table 9 (7, 22, 84). Further experience on the validity of this approach as well as on the criteria of interpretation of the results needs to be acquired before this method can be properly exploited in field applications.

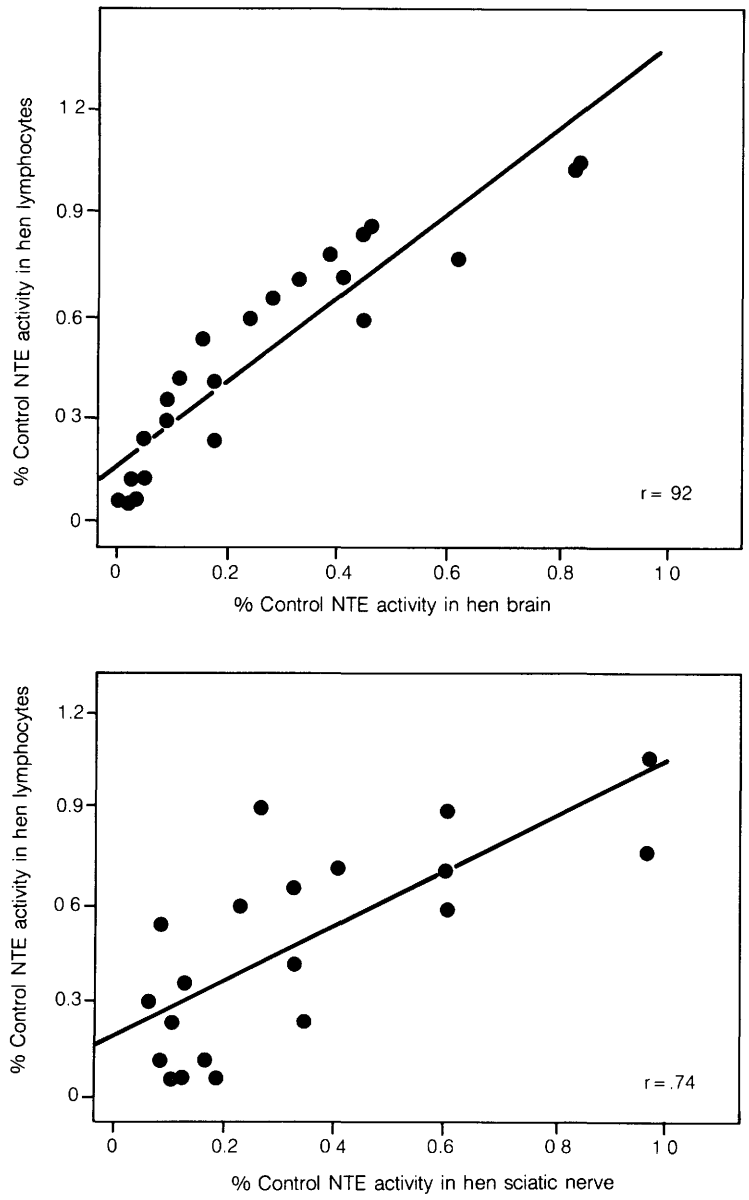


Fig. 16 Correlation between neurotoxic esterase activity in hen limphocytes and nervous system following DFP adminstration.

Table 8. Neurotoxic esterase activity following intramuscular administration of DFP in the hen

Time after DFP injection (a)	Neurotoxic esterase activity (b)			
	Lymphocyte	Brain	Spinal cord	Peripheral nerve
4 hr	4	2	7	15
8 hr	12	3	6	8
12 hr	10	4	8	14
24 hr	34	8	12	16
48 hr	39	15	17	25
72 hr	63	24	19	28
96 hr	76	33	26	41
7 day	91	42	37	44
9 day	70	45	44	72
14 day	107	79	71	97

(a) DFP dose: 1.4 mg/kg
(b) Percentage of baseline activity. Means of 4 animals. (from 55)

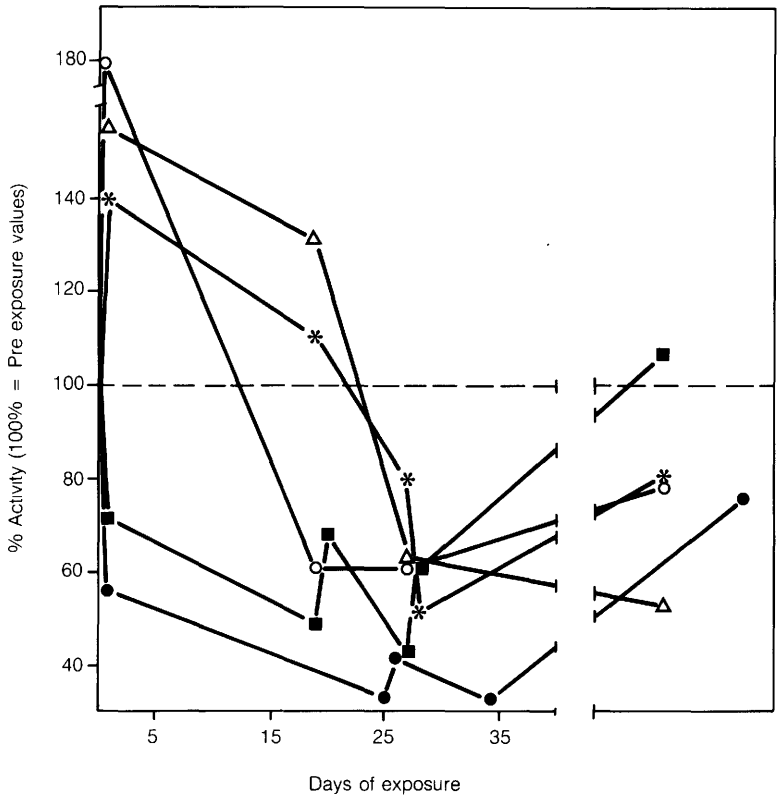


Fig. 17 Limphocytes'NTE activity in 5 workers during and after exposure to DEF. One hundred percent value for lymphocyte NTE activity is the baseline activity measured before the exposure in each subject. All values are expressed as percentage of that value. Last value for each worker was obtained three weeks after end of exposure (from 56).

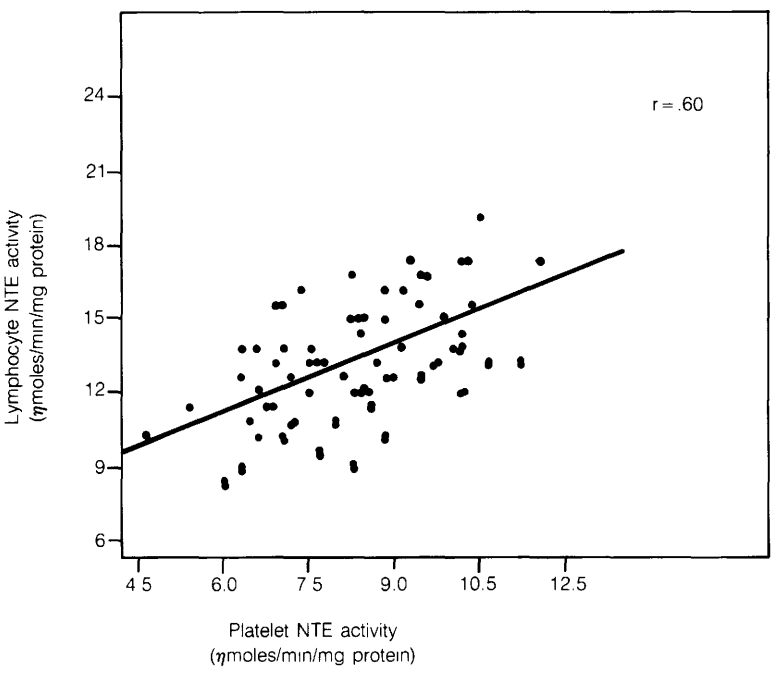


Fig. 18 Correlation between human lymphocyte and platelet neurotoxic esterase activity

Table 9. Reference values of lymphocyte and platelet NTE activity in different groups of subjects

Location	No of subjects	Lymphocytes (nmol.min^{-1}.mg prot^{-1})		Platelets (nmol.min^{-1}.mg prot^{-1})		Ref.
		Mean	SD	Mean	SD	
Baltimore, USA	68	13.2	2.4	8.4	1.5	7
Valtellina, Italy	35	11.9	3.0	8.5	1.9	84
Padova, Italy	108	11.5	2.5	—	—	22

Conclusions

The assessment of exposure to OP pesticides through biological indicators represents the only reliable way to evaluate OP exposure in many cases. The use of biological indicators, however, encounters some practical difficulties, related to the multiplicity of the OP compounds in use, the discontinuous and various modality of pesticide use in different contexts, and the paucity of knowledge and experience on the use of some of these biological indicators.

Among the indicators of internal dose, the urinary excretion of metabolites is the indicator of choice. For a few compounds, including parathion, parathion-methyl and fenitrothion, simple methods measuring phenolic derivatives in urine can be used. For the majority of the OP's, such methods are not available and the measurement of alkylphosphates in urine is the most suitable and appropriate procedure. The analytical method for measuring alkylphosphates in urine is rather sophisticated and laborious. Some OP's, including malathion, in addition to alkylphosphates give specific urinary metabolites.
The experience of use of indicators of internal dose for the OP compounds in man is very limited and interpretation of the results may be difficult.

The most important and widely used indicator of exposure to OP pesticides is the measurement of blood cholinesterase activity. Red blood cells and serum contain two different enzymes, with different specificity and biological variability.
The most appropriate for OP exposure monitoring is erythrocyte acetylcholinesterase (AchE). Inhibition of erythrocyte AchE shows a fair correlation with level of exposure as well as severity of intoxication in exposed subjects. Data on the relationship between AchE inhibition and urinary excretion of OP metabolites are available for very few OP pesticides.

Cholinesterase activity is related only to the acute toxicity of OP esters to the nervous tissue and does not inform about chronic or delayed neurotoxic effects.
Based almost exclusively on experimental animal studies, the determination of «Neuropathy Target Esterase» activity (NTE) in circulating lymphocytes or platelets has been proposed as a test to assess the human risk to develop delayed neurotoxic effects from OP's. This test however is still at an experimental phase and further studies are required to allow its utilization in man.

Very recent investigations have shown that certain OP pesticides may be inducers of liver microsomal enzymes or interfere with liver porphyrin metabolism in in vitro systems. If the occurence of these effects is confirmed also in man, the evaluation of microsomal enzyme induction and the determination of the pattern of urinary or plasmatic porphyrins could be of use as biological indicators of effect in the future.

Bibliographic references

General

Reviews on chemistry, metabolism, and toxicity of organophosphorous esters:

— REPORT OF A WORKING GROUP OF EXPERTS (1977)
Organophosphorus Pesticides. Criteria (Dose/effect Relationships) for Organophosphorus Pesticides. Published by Pergamon Press for the Commission of European Communities, EUR 5696.

— C.S. DAVIS & R.J. RICHARDSON (1980). Organophosphorous compounds.
In: P. Spencer & H. Schaumburg Eds., «Experimental and Clinical Neurotoxicology» William & Wilkins Publ., Baltimore, pp. 527-544.

— S.D. MURPHY (1980). Pesticides.
In: J. Doull, C.D. Klaassen, M.O. Amdur eds., Casarett and Doull's Toxicology, McMillan Publ. Co., New York, 357-408.

— W.J. HAYES Jr. (1980): Organic phosphorus pesticides.
In: Pesticides studied in man. W.J. Hayes Ed., William & Wilkins Publ., Baltimore, pp. 284-435.

— WORLD HEALTH ORGANIZATION. REGIONAL OFFICE FOR EUROPE (1980) - Toxicology of Pesticides. Health Aspects of Chemical Safety - Interim Document 9.

— UNITED NATIONS ENVIRONMENT PROGRAMME, INTERNATIONAL LABOUR ORGANIZATION, WORLD HEALTH ORGANIZATION (1984)
Environmental Health Criteria for Organophosphorous Insecticides - A General Introduction. International Programme on Chemical Safety, Internal Technical Report.

Specific

1. M.K. JOHNSON (1982). The target for initiation of delayed neurotoxicity by organophosphorous esters: biochemical studies and toxicological applications. Rev. Biochem. Toxicol. 4: 141-212.
2. M.K. JOHNSON (1975). Organophosphorous Esters Causing Delayed Neurotoxic Effects. Mechanism of Action and Structure/Activity Studies. Arch. Toxicol. 34: 259-288.
3. M.K. JOHNSON (1969). A phosphorylation site in brain and the delayed neurotoxic effect of some organophosphorus compounds, Biochem., J., 111: 487.
4. M.K. JOHNSON (1969). The delayed neurotoxic effect of some organophosphorus compounds. Identification of the phosphorylation site as an esterase, Biochem. J., 114: 711.
5. M.K. JOHNSON (1975). The delayed neuropathy caused by some organophosphorus esters: Mechanism and challenge, Crit. Rev. Toxicol., 3: 289.
6. M.K. JOHNSON (1977). Improved Assay of Neurotoxic Esterase for Screening Organophosphates for Delayed Neurotoxicity Potential. Arch. Toxicol. 37: 113-115.
7. M. MARONI, M.L. BLEECKER (1986). Neuropathy target esterase in human lymphocytes and platelets. Journal of Applied Toxicology, (6: 1-7).
8. A. MORETTO, A. FASSINA and M. LOTTI (1983). Neurotoxic Esterase in extranervous tissues of man. Presented at The Second International Meeting on Cholinesterases, Bled (Yugoslavia), sept. 17-21.
9. M. MARONI (1983). Recent acquisitions in biological monitoring of human exposure to chloroorganic and organophosphate pesticides. Proceedings of the international conference on environmental hazards of agrochemicals in developing countries, vol. 1, 345-371.
10. B.R. DUDEK, M. BARTH, L. GEPHART, J. HUGGINS and R.J. RICHARDSON (1979). Correlation of brain and lymphocyte neurotoxic esterase inhibition in the adult hen following dosing with neurotoxic compounds. Toxicol. Appl. Pharmacol. 48: A198.
11. C.S. DAVIS and R.J. RICHARDSON (1980). Organophosphorous compounds. In: P. Spencer and H. Schaumburg, eds, Experimental and Clinical Neurotoxicology. Baltimore: Williams and Wilkins, pp 527-544.
12. J.B. CAVANAGH (1973). Peripheral neuropathy caused by toxic agents. CRC Crit Rev Toxicol 2: 365-417.
13. M. LOTTI, C.E. BECKER, M.J. AMINOFF (1984). Organophosphate polyneuropathy: pathogenesis and prevention. Neurology, 34: 658-662.
14. J.P. MORGAN and P. PERROVICH (1978). Jamaica Ginger Paralysis. Forty-seven-Year Follow-up. Arch. Neurol. 35: 530-532.

15. P.L. BIDSTRUP, J.A. BONNEL and A.G. BECKETT (1953). Paralysis following poisoning by a new organic phosphorus insecticide (mipafox): report on two cases. Brit. Med. J. 1: 1068-1072.

16. C. XINTARAS, J.R. BURG, S. TANAKA, et al. (1978). Occupational Exposure to Leptophos and Other Chemicals. DHEW (NIOSH) Publication, 78-136.

17. R. HIERONS and M.K. JOHNSON (1978). Clinical and toxicological investigations of a case of delayed neuropathy in man after acute poisoning by an organophosphorous pesticide. Arch. Toxicol. 40: 279-284.

18. H. JEDRZEJOWSKA, K. ROWINSKA-MARCINSKA and B. HOPPE (1980). Neuropathy due to phytosol (Agritox): report of a case. Acta Neuropathol. (Berlin), 49: 163-168.

19. N. SENANAYAKE and M.K. JOHNSON (1982). Acute polyneuropathy after poisoning by a new organophosphate insecticide. N. Engl. J. Med. 306: 155-157.

20. K.W. JAGER, D.V. ROBERTS and A. WILSON (1970). Neuromuscular function in pesticide workers. Brit. J. Ind. Med., 27: 273-278.

21. D.V. ROBERTS (1977). A longitudinal electromyographic study of six men occupationally exposed to organophosphorus compounds. Int. Arch. Occup. Environ. Hlth 38: 221-229.

22. D. BERTONCIN, A. RUSSOLO, S. CAROLDI and M. LOTTI (1985). Neuropathy target esterase in human lymphocytes. Arch Environ Health 40: 139-143.

23. R.L. RODNITZKY, S.L. HARVEY and D.L. MICK (1975). Occupational exposure to organophosphate pesticides. A Neurobehavioural Study. Arch. Environ. Health, 30:98-103.

24. M.F. LEWIS et al. (1973). Behavioral changes from chronic exposure to pesticides used in aerial application: effects of Phosdrin on the performance of monkeys and pigeons on variable interval reinforcement schedules. Aerospace medicine, 44:290-293.

25. H.S LEVIN and L. RODNITZKY (1976). Behavioral effects of organophosphate pesticides in man. Clinical Toxicology, 9: 391-405.

26. D.R. METCALF and J.H. HOLMES (1969). EEG, psychological and neurological alterations in humans with organophosphorus exposure. Ann. NY Acad. Sci. 16: 357-365.

27. J.H. KOEMAN, P.M.H. DEBETS, J.J.T.W.A. STRIK (1980). The porphyrinogenic potential of pesticides with special emphasis on organophosphorus compounds. In: W.F. Tordoir and E.A.H. Heemstra-Lequin eds., «Studies in Environmental Science, vol. 7, Field Worker Exposure during Pesticide Application». Elsevier North/Holland, p. 157.

28. C. BOPP and B. KOSMINSKY (1975). Porfiria hépato-cutânea toxica. Med. Cut. I.L.A., 4: 271-280.

29. R. UPPAL, P.R. SHARMA and R.R. CHAUDHRURY (1982). Effect of Pesticide Exposure on Human Microsomal Enzyme Induction. Human Toxicol. 1: 155-158.

30. W.J. HAYES Jr (1982). Organic phosphorus pesticides. In: Pesticides studied in man. W.J. Hayes Jr. ed., William & Wilkins Publ., Baltimore, 284-435.

31. C. DONNINGER, D.H. HUTSON and B.A. PICKERING (1972). The oxidative dealkylation of insecticidal phosphoric acid triesters by mammalian liver enzymes. Biochem. J. 126: 701-7.

32. R.M. HOLLMGWORTH (1972). The dealkylation of organophosphorus triesters by liver enzymes. In O'Brien, R.D. and Yamamoto, I. (eds.): Biochemical Toxicology Insecticides. Academic Press, Inc., New York.

33. S.D. MURPHY (1969). Mechanisms of pesticide interactions in vertebrates. Residue Rev. 25: 201-221.

34. M. SU, F.K. KINOSHITA, J.P. FRAWLEY and K.P. DUBOIS (1971). Comparative inhibition of aliesterases and cholinesterase in rats fed eighteen organophosphorus insecticides. Toxicol. Appl. Pharmacol., 20: 241-49.

35. WORLD HEALTH ORGANIZATION (1982). Recommended health-based limits in occupational exposure to pesticides. WHO (World Health Organization), Technical Report Series 677, Geneva.

36. WORLD HEALTH ORGANIZATION (1967). Safe use of pesticides in public health: sixteenth report of the WHO Expert Committee on Insecticides. WHO Technical Report Series 356, Geneva.

37. D.P. MORGAN, H.L. HETZLER, E.F. SLACH and L.I. LIN (1977). Urinary excretion of paranitrophenol and alkyl phosphates following ingestion of methyl or ethyl parathion by human subjects. Arch. Environ. Contam. Toxicol., 6: 159-173.

38. M. NOSAL and A. HLADKA (1968). Determination of the exposure to fenitrothion (O,O-Dimethyl-O [3-methyl-4-nitrophenil] thiophosphate) on the basis of the excretion of p-nitro-m-cresol by the urine of the persons tested. Intern. Arch. Gewerbepathol. Gewerbehyg., 25: 28-38.

39. T. SHAFIK, D.E. BRADWAY, H.F. ENOS, A.R. YOBS (1973). Human exposure to organophosphorous pesticides. A modified procedure for the gas-liquid chromatographic analysis of the alkyl phosphate metabolites in urine. J. Agr. Food Chem. 21: 625-629.

40. S.J. REID, R.R. WATTS (1981). A method for the determination of dialkyl phosphate residues in urine J. Anal. Toxicol., 5.

41. D.Y. TAKADE, J.M. REYNOLDS, J.H. NELSON (1979). 1-(4-Nitrobenzyl)-3-(4-tolyl)triazene as a derivatizing reagent for the analysis of urinary dialkyl phosphate metabolites of organophosphorus pesticides by gas chromatography. J. Agr. Food Chem., 27: 746.

42. R.S. MURPHY, F.W. KUTZ, S.C. STRASSMANN (1983). Selected pesticides residues or metabolites in blood and urine specimen from a general population survey. Environ. Hlth. Persp. 48: 81-86.

43. J.C. GAGE (1967). The significance of blood cholinesterase activity measurements, Residue Reviews; 18: 159-167.

44. G. VOSS, K. SACHSEE (1970). Red Cell and Plasma Cholinesterase Activity in Microsamples of Human and Animal Blood Determinated Simultaneously, by a modified Acetylthiocholine/DTNB Procedure. Toxic. Appl. Pharmacol. 16: 764-772.

45. K. WILHELM and V. BRADAMANTE (1980). Blood cholinesterase activity in workers exposed to cholinesterase. A ten-year follow up Arh. Hig. Rada Toksikol. 31: 109-12 4.

46. G. LOCATI, G. CAVAGNA (1968). Valori normali di attività colinesterasica ematica determinati con metodo titrimetrico automatico. Med. Lav. 59: 516-521.

47. K.B. AUGUSTINSSON (1955). The normal variation of human blood cholinesterase activity Acta Physiol. Scand. 35: 40-52.

48. J. PEH, R.A. Mc CANCE (1949). Enzyme activities in the blood of infants and adults Biochem. J. 45: 464-467.

49. K.M. KUTTY (1980). Review: Biological function of cholinesterase, Clinic. Biochem., 13: 239-243.

50. ANDO MITSURU, SHOTA HIROSAKI, KENJI TAMURA, TOSHIMITSU TAYA (1984). Multiple Regression Analysis of the Cholinesterase Activity with Certain Physiochemical Factors. Environm. Res. 33:96-105.

51. J.A. RIDER, J.L. HODGES, J. SWADER and A.D. WIGGINS (1975). Plasma and red cell cholinesterase in 800 «healthy» blood donors. J. Lab. Clin. Med., 50: 376-383.

52. J.H. WILLS (1972). The measurement and significance of changes in the cholinesterase activities of erythrocytes and plasma in man and animals C.R.C. Crit. Rev. Toxicol. 2: 153.

53. WORLD HEALTH ORGANIZATION - REGIONAL OFFICE FOR EUROPE. Epidemiological study on the health effects of exposure to organophosphorus pesticides. Core protocol, March 1985.

54. R.L. ZIELHUIS (1972). Epidemiological Toxicology of Pesticide Exposure. Report of an International Workshop. Arch. Environ. Health. 25: 399-405.

55. M.L. BLEECKER, M. MARONI, M. SEPANSKI (1983). A biological marker for organophosphate exposure: neurotoxic esterase activity in human lymphocytes and platelets. In: Development in the Science and Practice of Toxicology, A.W. Hayes, R.C. Schnell and T.S. Mija eds., Elsevier Science Publishers B.V.

56. M. LOTTI, C.E. BECKER, M.J. AMINOFF, J.E. WOODROW, J.N. SEIBER, R.E. TALCOTT and R.J. RICHARDSON (1983). Occupational exposure to the cotton-defoliants DEF and Merphos. J. Occup. Med. 25: 517-522.

57. R. DERACHE (1977). Organophosphorus Pesticides. Criteria (Dose/Effect Relationship) for Organophosphorus Pesticides. Report of a Working Group of Experts prepared for the Commission of the European Communities, Directorate General for Social Affairs, Health and Safety Directorate. Commission of the European Communities by Pergamon Press, EUR 5696.

58. J.A. RIDER, J.I. SWADER and E.J. PULETTI (1971). Anticholinesterase toxicity studies with methyl parathion, Guthion, and Phosdrin in human subjects. Fed. Proc. 30: 443.

59. J.A. RIDER, J.I. SWADER and E.J. PULETTI (1972). Anticholinesterase toxicity studies with Guthion, Phosdrin, Di-Syston, and Trithion in human subjects. Fed. Proc. 31: 520.

60. C.G. HUNTER, J. ROBINSON, C.T. BEDFORD and J.M. LAWSON (1972). Exposure to chlorfenvinphos by determination of a urinary metabolite. J. Occup. Med., 14: 119-122.

61. E.M. LORES, D.E. BRADWAY and R.F. MOSEMAN (1978). Organophosphorus pesticide poisonings in humans: Determination of residues and metabolites in tissues and urine. Arch. Environ. Health, 33: 270-276.

62. E.M. LORES, G.W. SOVOCOOL, R.L. HARLESS, N.K. WILSON and R.F. MOSEMAN (1978). A new metabolite of chlorpyrifos: Isolation and identification. J. Agric. Food Chem., 26: 118-122.

63. T.B. GRIFFIN, F. COULSTON, D.D. McCOLLISTER (1976). Studies of the relative toxicities of chlorpyrifos and chlorpyrifosmethyl in man. Toxicol. Appl. Pharmacol. 37: 105.

64. J.A. RIDER, H.C. MOELLER, E.J. PULETTI and J.I. SWADER (1969). Toxicity of parathion, Systox. octamethyl pyrophosphoramide and methyl parathion in man. Toxicol. Appl. Pharmacol., 14: 603-611.

65. FAO/WHO (1967). Evaluation of Some Pesticide Residues in Food. Monograph prepared by the Joint Meeting of the FAO Working Party and the WHO Expert Committee on Pesticide Residues, which met in Geneva, 14-21 November 1966. WHO/Food Add./67.32 Geneva.

66. J.E. DAVIES, A. BARQUET, V.H. FREED, R. HAQUE, C. MORGADE, R.E. SONNEBORN and C. VACLAVEK (1975). Human pesticide poisonings by a fat-soluble organophosphate insecticide. Arch. Environ. Health, 30: 608-613.

67. D.H. HUSTON and E.C. HOADLEY (1972). The comparative metabolism of (^{14}C-vinyl) dichlorvos in animals and man. Arch. Toxicol., 30: 9-18.

68. J.A. RIDER, H.C. MOELLER and E.J. PULETTI (1967). Continuing studies on anticholinesterase effect of methyl parathion, initial studies with Guthion, and determination of incipient toxicity level of dichlorvos in humans. Fed. Proc., 26: 427-x.

69. J.A. RIDER, H.C. MOELLER, E.J. PULETTI and J. SWADER (1968). Studies on the anticholinesterase effects of methyl parathion, Guthion, dichlorvos, and Gardona in human subjects. Fed. Proc., 27: 597.

70. E.F. EDSON, K.H. JONES and W.A. WATSON (1967). Safety of dimethoate insecticide Br. Med. J., 4: 554-555.

71. D.M. SANDERSON and E.F. EDSON (1964). Toxicological properties of the organophosphorus insecticide dimethoate. Br. J. Ind. Med., 21: 52-64.

72. D.E. BRADWAY and T.M. SHAFIK (1977). Malathion exposure studies. Determination of mono- and dicarboxylic acids and alkyl phosphates in urine. J. Agric. Food Chem., 25: 1342-1344.

73. H.C. MOELLER and J.A. RIDER (1962). Plasma and red blood cell cholinesterase activity as indications of the threshold of incipient toxicity of ethyl-p-nitrophenyl thionobenzenephosphonate (EPN) and malathion in human beings. Toxicol. Appl. Pharmacol., 4:123-130.

74. D.L. BULL (1968). Metabolism of O,O-dimethyl phosphorodithioate S-ester with 4-(mercaptomethyl)-2-methyl-Δ^2-1,3,4-thiadazolin-5-one (Geigy GS-13005) in plants and animals. J. Agric. Food Chem., 16: 610-616.

75. FAO/WHO (1973). 1972 Evaluations of Some Pesticide Residue in Food. Monograph prepared by the Joint Meeting of the FAO Working Party of Experts on Pesticide Residues and the WHO Expert Committee on Pesticide Residues that met in Rome from 20 to 28 November 1972. WHO Pesticide Residue Series, No. 2, Geneva.

76. J.H. HOLMES, H.G. Jr. STARR, R.C. HANISCH and K.N. VON KAULLA (1974). Short-term toxicity of mevinphos in man. Arch. Environ. Health, 29: 84-89.

77. J.A. RIDER, E.J. PULETTI and J.I. SWADER (1975). The minimal oral toxicity level for mevinphos in man. Toxicol. Appl. Pharmacol., 32: 97-100.

78. H.R. WOLFE, W.F. DURHAM and J.F. ARMSTRONG (1970). Urinary excretion of insecticide metabolites: Excretion of para-nitrophenol and DDA as indicators of exposure to parathion. Arch. Environ. Health 21: 711-716.

79. E.F. EDSON (1964). No-effect levels of three organophosphates in the rat, pig and man. Food Cosmet. Toxicol., 2: 311-316.

80. J.A. RIDER, J.I. SWADER and E.J. PULETTI (1970). Methyl parathion and Guthion anticholinesterase effects in human subjects. Fed. Proc., 29: 349.

81. B. HOLMSTEDT, I. NORDGREN, M. SANDOZ and A. SUNDWALL (1978). Metrifonate, summary of toxicological and pharmacological information available. Arch. Toxicol., 41: 3-29.

82. R.P. MOODY, C.A. FRANKLIN, D. RIEDEL, N.I. MUIR, R. GREENHALGH, A. HLADKA (1985). A new GC on-column methylation procedure for analysis of DMTP (O,O-dimethyl phosphorothioate) in urine of workers exposed to fenitrothion. J. Agric. Food Chem., 33: 464-467.

83. J.F. KRAUS, D.M. RICHARDS, N.O. BORHANI, R. MULL, W.W. KILGORE, W. WINTERLIN (1977). Physiological response to organophosphate residues in field workers. Arch. Environ. Cont. Toxicol., 5: 471-485.

84. M. MARONI, A. COLOMBI, R. GILIOLI, M.L. BLEECKER, L. VILLA, V. FOA' (1985). Reference values for lymphocyte and platelet NTE activity in American and Italian populations. Presented at the Second International Symposium on Neurobehavioural Methods in Occupational and Environmental Health, Copenhagen, August 6-9, 1985.

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