POISONING BY HOME PRODUCTS— CHEMICAL AND BIOLOGICAL METHODS OF DIAGNOSIS

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INTRODUCTION

At the present stage of progress by chemical industry it may appear needless to fear from the toxic nature of household products so common through their use; and yet acute intoxication fairly often occurs especially in children (one to four years old) who escape their parents eye and swallow anything they find at home. The seriousness of intoxication by home products can be assessed from figures for the year 1965–66 during which 9830 calls were received at the Poison Centre in Paris and 238 children were admitted to hospital (cf. *Tables 1* and *2*). From the medical practitioners' point of view these accidents are mostly mild, especially when they are due to intaking of solids, but more dangerous when solvents or cleansing fluids are swallowed. With teenagers and adults the acute intoxication results from a suicide attempt or carelessness.

Table 1. Data on the poisoning in children by home products in Paris during the year 1965–66 (in hospitalized cases)

Poisons	*Monday*	*Tuesday*	*Wednesday*	*Thursday*	*Friday*	*Saturday*	*Sunday*	*Total*
Medical preparations	19	15	19	23	12	16	12	116
Other home products	27	20	11	18	19	15	12	122
Total	46	35	30	41	31	31	24	238

Physicians and biologists are faced with the problem of treating intoxication which they do differently except for the first stage, viz. detection. Analytical methods used in clinical toxicology, and general analytical chemistry methods are the same (quantitative and qualitative), except for two points: limits due to human characteristics of samples, and delay necessary for giving useful data.

Samples are generally blood, urine and seldom fat or organ-fragments. In the case of fat or organ-fragments there is, in addition, an initial stage of extraction of the toxic substance. This is either accomplished by destruction of organic matter (for analysis of non-volatile minerals), or after purification by mild procedures like adsorption for analysis of organic chemicals. Time taken for the detection of toxic substance must not exceed

Table 2. An analysis of the calls received at the poison centre, Paris (Fernand Widal Hospital) during the year 1965–66. Total number of calls 9830.

COMBUSTIBLES	
Town gas (CO)	86
Butane	15
Metaldehyde	62
Liquid petroleum products	172
Total:	335
RATICIDES	
Anticoagulants	45
Chloralose	41
Strychnine	30
Crimidine	4
Powders containing phosphorus	3
Others	55
Total:	178
INSECTICIDES	
Parathion	26
Diméthon méthyle	9
Other organophosphates	10
DDT	31
HCH	10
Lindane	41
Dieldrine	14
Chlordane	1
Nicotine	4
Dinitro-*o*-cresol	2
Dichlorophenoxyacetic acid	2
Others	42
Total:	209
HOUSEHOLD AND FARM PRODUCTS	
Pentachlorophenol	10
Weed eradicators	11
Various fertilizers	65
Cosmetics	223
Powder and liquid detergents	276
Paradichlorobenzene	247
Caustic soda	66
Surface water	309
Turpentine oil	30
Fluorides	53
Weed eradicators	43
Oxalates	75
Tobacco	35
Others	403
Total:	1537
Animal food	305
Vegetable food	3
INDUSTRIAL PRODUCTS	1096

a few hours, if the biologist means to give useful information to the physician.

Laboratory responsibility is very peculiar. Analysis certifies the eventual correlation between illness and toxicity, and in medico-legal cases the presence of a poison furnishes the first and foremost evidence. Whichever is the cause of poisoning (therapeutic, industrial or criminal), even if damage is not voluntary, common law imposes at least compensation for the ill effects which follow intoxication. This aspect, of great importance, shows the permanence of relationship between toxicology and forensic medicine, which require from the laboratory an exceptional security of the methods used, an extreme prudence in statement and interpretation of results.

The oblivion of these main ideas is the cause of many errors, full of many regrettable consequences. We have to recognise that clinical toxicology requires a great effort from the chemist. Besides common intoxications like those due to hypnotics or carbon monoxide which correspond to a large routine work up, sporadic intoxications need special apparatuses and reagents for each chemical. Group reactions are known and permit a real economy of means. But they are not numerous, which explains the big difference between analytical work of agricultural chemists, and that of clinical toxicologists.

This paper's aim is to explain to the first ones the problems interesting the second ones, and in return, to propose to physicians refined methods they cannot very easily know because such reports are not published in medical journals.

We know that there are not many hospitals which have clinical toxicology departments but it is a beginning, and the ever increasing Poison Centres, are indicative of a normal evolution. We shall consider clinical aspects only as examples intended for illustration of difficulties inherent to this part of medicine.

DIAGNOSIS OF ACUTE INTOXICATIONS

As we have been requested, we shall limit this paper to the diagnosis of intoxication by pesticides, and some other great groups of chemicals; the number of products used effectively exceed 400, whereof 200 are organochlorides and 130 are organophosphates. We shall not speak of carbon monoxide poisoning and of results of techniques of hyperbaric oxygen, studied elsewhere. The main pesticides used in France which cause human intoxication are listed in *Tables 2* and *3*.

Table 3. Main pesticides used in France and their degrees of toxicity

Pesticide	*Acute toxicity* (LD 50) *Per os*	*Skin toxicity*	
	ORGANOCHLORIDES		
Aldrin	30–90	100	(constant according to source)
Dieldrin	45	80	(constant according to source)
Endrin	3–40	5–15	(varies as the source)
DDT	300	2500	
Lindane	90	1000	
Heptachlore	150	250	
Toxaphène	90	1000	
	ORGANOPHOSPHORUS COMPOUNDS		
(Ethyl) parathion	6	15–30	
Methyl parathion	25	65	
Malathion	1000	4500	
Diazinon	100	600	
	DIVERS		
DNP DNOC	100	600	
2,4D	50		(dog)

Table 4. Electroencephalographic disorders and cholinesterase levels during intoxication by organophosphates and organochlorides (F. Widal Hospital)

Case No.	*Mode of intoxication*	*Clinical state of the patient*	*Period*	*EEG*	*Cholinesterases* Pl	H	*Tt*	*Evol.*
		ORGANO PHOSPHORUS INSECTICIDES						
22	Taken orally (suicide)	Conscious myosis respiratory impairment	2 days	Very near normal, alpha labile	0·24	0·40	+	cured
34	Taken orally (accidental)	Myosis	2 days	Desynchronized approaching normal théta rythme abundant maximum in parasagittal	—	—	+	cured

Table 4 continued on p. 154

Table 4—continued

Case No.	*Mode of intoxication*	*Clinical state of the patient*	*Period*	*EEG*	*Cholinesterases* Pl	H	*Tt*	*Evol.*
306	By inhaling (sub-acute)	Conscious	12 days 20 days	Period of discretion	0·39	0·55	+	cured
831	Taken orally (suicide)	Extreme state of coma; Myosis; temp. 36·2	2 days	No response	0·18	—		died
831	Taken orally (suicide)	Coma stage II Coma (watchful) Obnubilation Conscious	4 days 5 days 7 days 15 days	Bradyrythmy Absence of reactivity Bradyrythmy Reactivity —N N N	0·13 0·13 0·12	0·46 0·58 0·40	+ + +	cured
		MIXED ORGANOPHOSPHORUS + ORGANOCHLORIDE INSECTICIDES						
43	By inhaling (sub-acute) Organo-chlorides + organo-phosphates (?)	Visual trouble nystagmus désorienta-tion dizziness	?	N (alpha labile)	0·81	0·98	+	Névrite rétro-bulbaire
188	By inhaling OCl + OP	Conscious headache, dizziness	3 days	N	0·93	0·44	+	cured
752	By inhaling OCl + OP + 2,4D + nicotine	Conscious	5 days 15 days	alpha slow unstable beta bitemporal at maximum left N	0·14	0·11		cured
		ORGANOCHLORIDE INSECTICIDES						
468	Kerosene (suicide)	Obnubilation	2 days	Alpha slow non-reactive points slow rythms changing to rapid ones	+			cured
1065	Aldrine taken orally (suicide) 6g?	Conscious	2 days	Approaching normal rapid rythms; medical reaction	+			cured
1110	By inhaling	Conscious	2 days	Normal	0·10	0·34		cured
1109	By inhaling	Conscious	2 days	Irregular	0·20	0·08		cured

ANALYTICAL METHODS AND IDENTIFICATION

Toxicological diagnosis of acute intoxication is still directly connected with forensic diagnosis of death by poisoning. It does not need very fine techniques and can be realised with limited means. First applications to technical toxicology have been easy but quick modifications are necessary, due to the evolution of analytical procedures, and of poisoning circumstances (suicide for example is realised by numerous substances, different from usual criminal poisoning). As an example, the extraction of the intoxicant has been for a long time the application of Stas–Otto procedure with or without modifications. Now, the treatment by acid-ether or alkaline chloroform still used for barbiturates or alkaloids, may cause degradation of a great number of modern organic chemicals. Therefore there is an increase in the use of neutral solvents, polar or non-polar, e.g. chlorinated solvents, dimethylformamide, acetonitrile, and dimethylsulphone. The methods of analysis of pesticides use only solvent partition and, during many years, emphasis has been laid on the choice of solvents, loss of pesticides incorporated in test-samples, variations due to biological samples—fatty or not, aqueous or not, destruction of pesticides even by polar solvents alone (dithiocarbamates). Some physical procedures are still used, e.g. steam distillation and sublimation. Sublimation points out in addition the probability of chemical modification during gas-chromatography (e.g. thermal isomerization of endrine).

Organochloro pesticides

Specific methods to be used in the case of acute intoxication can be deduced from macro-methods in use for analysis of commercial products. The extraction of gastric lavage fluids by non-polar solvents (hexane) is very effective and permits a quick purification by mild evaporation, redissolution.

Among the general methods, total organic chloride determination (Stepanow modified), i.r. spectroscopy (dieldrine group), polarography (isomer of HCH is the only one reducible isomer) can be used; colorimetric determination of DDT by pyridrine, alkaline xandhydrol (Stiff and Castillo), of aldrine (action of phenylazide, coupling with diazonium salt of 2,4-dinitroaniline) (Danish and Lidov) can be used as well.

Toxic doses are very high (*n* grammes) and the results shall be positive both on suspicious products, and on digestive contents. But absorption is slow and levels in blood and tissues need the same methods as those used during subacute or even chronic intoxications [organic chloride determination and gel permeation chromatography (GPC) separation or thin-layer determination], which shall be described later in this communication.

ORGANOPHOSPHATES AND MISCELLANEOUS PESTICIDES

In this group, macro-methods have been described for commercial products but there is no general procedure. Each chemical is determined through by-products of hydrolytic reactions (malathion by complex of phosphodithiazoate of Na*O*,*O*,dimethyline), or by non-specific radical identification (reduction of nitro in amine for parathion, diazotization and

coupling with *N*-naphthylethylene-diamine (classical method of Everell and Norris which differentiates parathion from non-reactive *p*-nitrophenol). same remark applies to other pesticides known in clinical toxicology.

Consequences are troublesome for a clinical toxicologist: either he is satisfied with the patient's or his entourage's interrogatory, or during hours, the biologist will repeat extractions and reactions without prejudice (and without pride because most of these acute intoxications are benign or easier detected by biochemical reactions; for instance, cholinesterase inhibition by organophosphates).

Application to human patients

Organophosphates can be detected in the digestive tract. But the general method for volatile phosphorus fails in the case of blood, urine and tissues. This can be explained as due to the speed of hydrolysis of organophosphates by *B. esterases* (Aldridge). We had some difficulty in determining the rates of hydrolysis of parathion in human blood—much less active than rabbit's blood, but we have had good results using manometric methods and phosdrine (cf. *Table 5* and *Figure 1*).

Table 5. Results of phosdrine hydrolysis by blood serum (mean values)

Spontaneous hydrolysis of phosdrine:	8 μl. CO_2 by $\frac{1}{2}$h
Spontaneous hydrolysis of human serum:	2·5 μl. CO_2 by $\frac{1}{2}$h
Spontaneous hydrolysis of rabbit serum:	4·4 μl. CO_2 by $\frac{1}{2}$h
Hydrolysis of phosdrine in the presence of rabbit serum:	85 μl. CO_2 by $\frac{1}{2}$h
Hydrolysis of phosdrine in the presence of human serum:	19 μl. CO_2 by $\frac{1}{2}$h
Dose: 3·5 ml	
CO_3 HNa	0·031 M
NaCl	0·162 M
Gelatin	0·1%
pH	7·73 at 37°
Gases	CO_2 5%; N_2 95%
Serum	0·5 ml
Phosdrine	10 mg/ml
Equilibrium	10 min
Survey	30 min

Enzymatic hydrolysis was found to be different in different human patients, and we could connect this with the degree of hepatic cellular deficiency. A quick evaluation of the speed of hydrolysis demonstrates that destruction can be as high as a fraction of a gram per hour which explains the theoretical and practical impossibility of direct demonstration of organophosphate poisoning, a few cases of rapid death excepted.

For parathion detection by a metabolite, e.g. *p*-nitrophenol is possible but toxic absorption is limited and urinary excretion rather low. Results of 10–40 mg/l. of *p*-nitrophenol in urine are arguments for acute intoxications by parathion and analogue (cf. *Figure 2*).

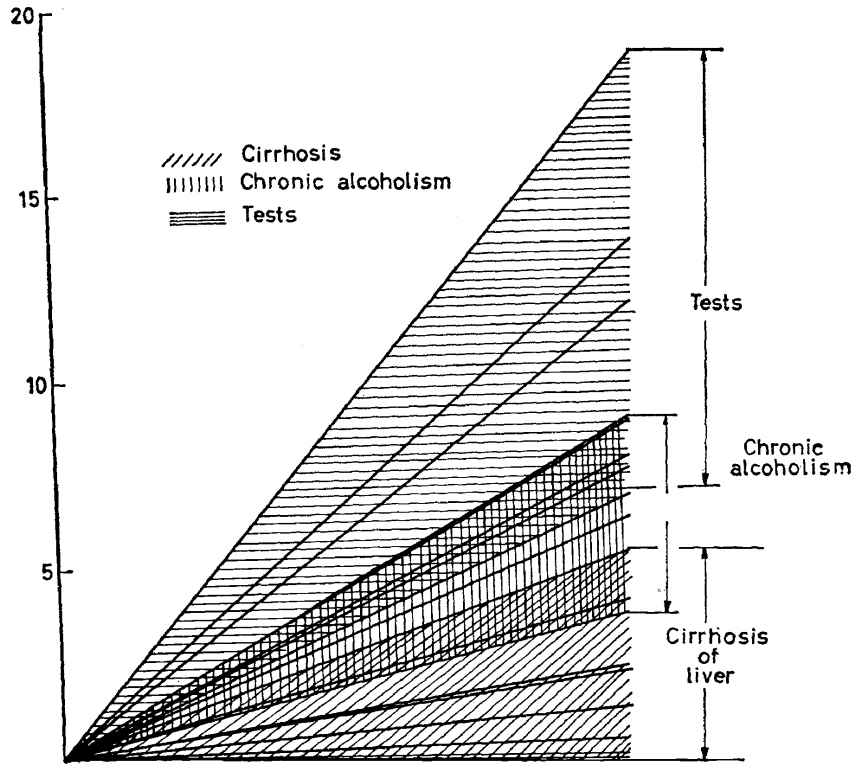

Figure 1. Speed of hydrolysis of phosdrin by blood sera

For organophosphates biological methods look much more accurate until progress in detection by very sensitive methods (to be seen with chronic intoxications) permit taking into consideration very small quantities of organophosphate.

Biochemical detection. Organophosphates are cholinesterases inhibitors and this is the main explanation of their toxicity. Actually, the inhibition of cholinesterases has the advantages of bringing out during a long time (many hours) biochemical result of intoxication.

Although biochemists have demonstrated the complexity of cholinesterase their possible classification is true (ACH cholinesterases) and pseudo cholinesterases, existence of congenital abnormalitites and of many isoenzymes, global methods are generally used. Numerous electrometric methods apply the technique used by Michel.

In the case of acute intoxication by organophosphates, a constant figure is found: *low levels of cholinesterases in plasma* (pseudo cholinesterases) *and red cells* (true acetylcholinesterases). Colorimetric methods and paper tests are used also, but they are less sensitive. We have registered during one very severe intoxication relative discrepancies between paper test and electrometric data. We noticed after a complete inhibition [ΔpH/h 0·03 (plasma), 0·18 (hematies)] a sharp restoration to [0·48 (P)–0·78 (H)]

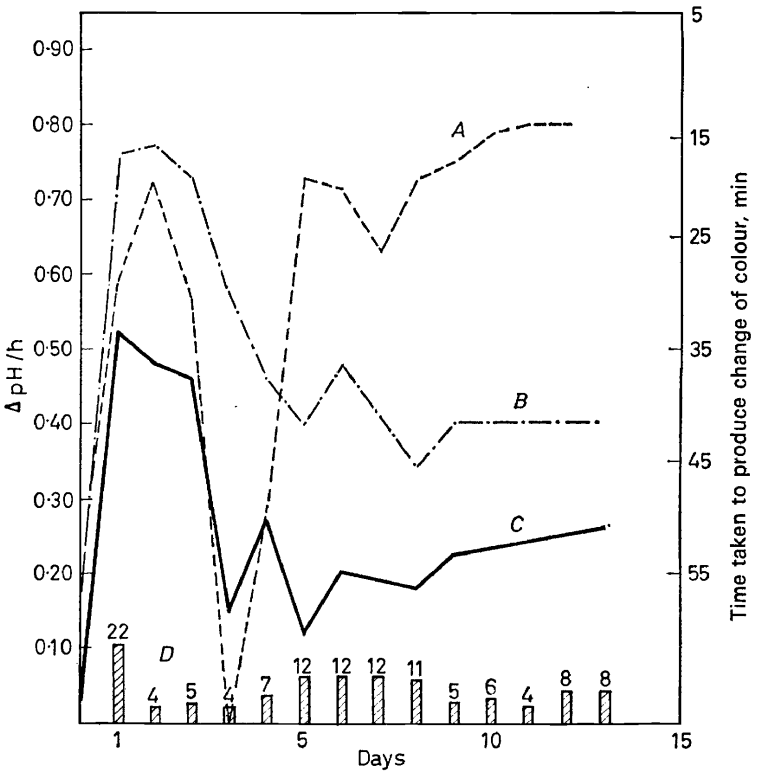

Figure 2. Comparison between paper-cholinesterase test and electrometric method in the case of acute intoxication by parathion

A, Paper test
B, Cholinesterases of erythrocytes
C, Cholinesterases in plasma
D, Contrathion (1 = 200 mg i.v.)

after injection IV of a large amount of antidote (contrathion, 4·4 g the first day). But doses of 1 g per day have not been sufficient to maintain this good result, and during more than ten days the level remained low [from 0·12 (P), 0·26 (H) to 0·32 (P), 0·52 (H)]. At this time, we did not know exactly the range of toxicity of this antidote, which is very low in fact.

Diagnosis value

Only nearly complete inhibition remains an excellent argument for acute intoxication and the very great resistance of these enzymes to complete hydrolysis permits late diagnosis on human body.

A moderate lowering of cholinesterases does not lead to diagnosis. We did find it in normal people [(one out of twenty has levels of 0·42 (P), 0·52 (H)] and by diseased, non-toxic (hepatic insufficiency), [down to 0·26 (P), 0·32 (H)] or toxic (fluorides), [down to 0·24 (P), 0·40 (H)].

Complete inhibition has a very great value in clinical toxicology for the variability of toxic symptoms and peculiarity of the first ones. If the

signs of acetylcholin-non-destruction are important, one must not forget that some OP give other anomalies, e.g. astheny and headache, and that many digestive symptoms are not specific of any intoxication.

Moreover, the use of mixtures of pesticides, the association of home products or solvent poisoning, make the work of biologists very difficult; *therefore the necessity of determination of blood cholinesterases as routine tests in clinical toxicology*. This is a very useful technique to eliminate probability of poisoning and micro-methods have been described for very small samples (Truhaut).

MISCELLANEOUS

Metallic intoxication

Very dangerous acute intoxications are due to *arsenic derivatives*. In this case Marsh–Cribier methods give good results. Urinary elimination passes some milligrams per litre, blood level 0·4 mg/l. It is useless to insist on the quick identification of arsenic in gastric lavage. Even radiography of abdomen can make appearance in an x-ray-absorbing substance in the stomach of the intoxicated.

Lead. Detection by dithizone is easy too.

Alkaloids

Nicotine. Extraction with chloroform–alkali followed by paper or thin-layer chromatography is a very successful procedure. Nicotine can be identified after purification by steam distillation in a strongly alkaline medium. Alkaloid precipitation and u.v. spectroscopy are useful (*Figure 3*).

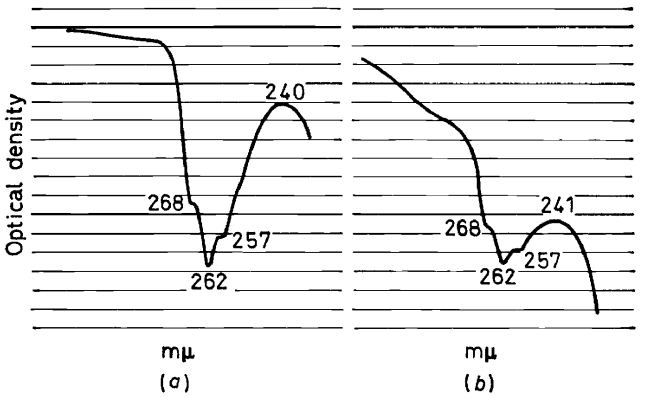

Figure 3. Ultraviolet spectra of (*a*) vomit distillate, (*b*) nicotine

But u.v. absorption has the great disadvantage of being poorly specific. Thin-layer chromatography is very useful for the identification of alkaloids in biological samples.

Strychnine. We observed some acute intoxications by strychnine. Detection in urine was very easy (sulphuric acid, potassium dichromate) and positive during many hours (Bourdon).

Chlorinated solvents

Industrial solvents as household products can be used without control and may cause many accidents. Insecticides are often sprayed after dissolution in solvents more or less dangerous.

Trichlorethylene, more seldon carbon tetrachloride, can be easily identified by Fujiwara reaction, and we may follow trichloride metabolic compounds in blood and urine especially trichloracetic acid (*Figure 4*). This

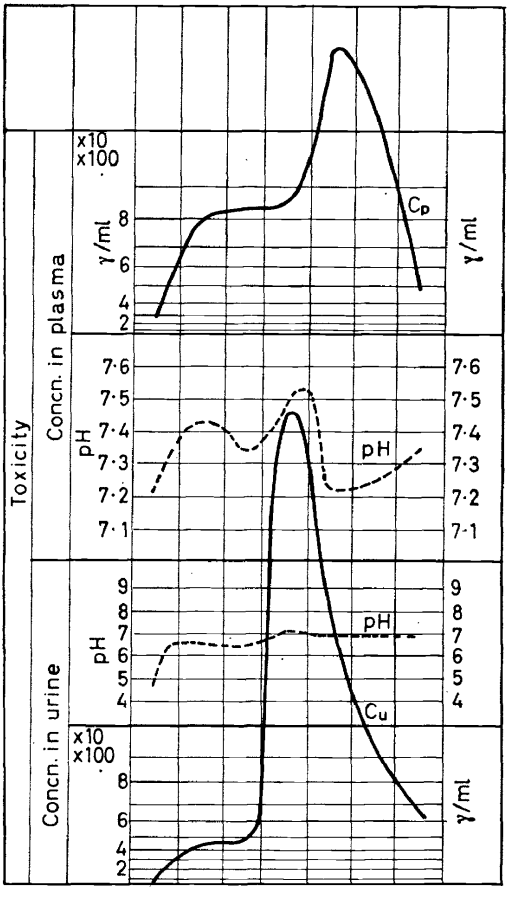

Figure 4. Acute intoxication by trichloroethylene (Trichlor metabolites)

[C_u = concentration in urine; C_p = concentration in plasma]

method lets appear some differences between colours of by-products, but real identification needs a more accurate procedure. One point of interest of this part of clinical toxicology, is the small amount of metabolite excreted in urine, compared with the dose absorbed. It explains the need of artificial respiration (without condensator) to detoxicate patients poisoned by solvents.

Other substances

Chloralose is rarely implied. In one lethal poisoning, blood level of chloralose was 50 mg/l.

Metaldehyde. Very dangerous, often eaten by children who mistake it for sugar, it can be easily identified by Schiff or guaiacol reaction on the gastric fluid. The reason of its toxicity looks still very obscure and there is a curious lack of biological theories.

Fluorides—fluoroacetates. Used as ratkiller, fluoracetate (sodium) is very toxic for humans. Acute intoxication by fluoride has been seen with anti-rut. In one acute intoxication by fluoride (about 4·4 g) levels of 6·63 mg/l. in blood, 6·86 mg/l. in liver were found (Dodimal).

Crimidine (2-chloro-5-methyl-6-dimethylamino pyrimidine). We received a call for a probable crimidine poisoning. Although this chemical is highly toxic, no convulsive symptoms appeared, and we did not try to use Freytag reaction (blue fluorescence in u.v. after reaction on paper with alkaline resorcinol).

Coumarinics. Their use is growing very quickly; their acute toxicity for man being very low, we seldom observe intoxicated people and we have no other data than those obtained during anticoagulant therapy. Physical dosage method—u.v. spectroscopy can be used for identification. Physicians are more interested in antivitamin K activity: coagulability test, Quick-time survey. Factor VII reduction is the first and most important plasmatic anomaly.

Nitrophenols–nitrocresols. During acute intoxications, these chemicals can be demonstrated in urine by reaction of Derrien (not very sensitive), with Mayer reaction, which detects at the same time nitro and amino derivatives.

Showing a good example of dissociation of physician interest from chemical analyst work when poisoning is regularly benign, we did not find any recent work on hypochlorites, cresols, non-chlorinated solvents (gasoline, kerosene), *p*-dichlorobenzene, naphthalene, and many other house-products, whose toxicity is low when considering doses usually taken by children.

Nevertheless, human acute intoxication has the value of a dramatic experience whose teaching is not to be neglected. It is a rare event to be caught at once, thanks to long preparation.

DIAGNOSIS OF SUBACUTE AND CHRONIC INTOXICATIONS

Quite different is chemical analysis applied to toxic impregnation (whether really dangerous or not). Methodology looks still uncertain and clinical use of results highly hazardous. Certainly progress in clinical toxicology is very recent, or chemist-isolated, so that they hardly benefit by new apparatus and methods proposed in papers concernin gagriculture or food. However, the possibilities of analytical chemistry are so wide open that we shall only speak of them and their medical application.

Atomic absorption spectroscopy. Reserved to mineral toxicology, atomic absorption spectroscopy (AAS) is a new method whose possibilities are limited by

characteristics of emission spectrum, by some interferences, especially with biochemicals and with phosphates (Rousselet).

Sensitiveness is described as 5h ppb for Li, Na, Mg, Ca, Zn, Rh, which interest us for *Lithium* and *Zinc* (especially after use of dithiocarbamates); 0·05 ppm with K, Cr, Mn, Fe, Co, Ni, Cr, Be, Sr, Ag, Cs which can be useful for *Mn*; 0·5 ppm with *Hg*, Ti, *Pb*; so that AAS can be used during acute poisoning, but remains at the practical limit of utilisation for chronic intoxications with these important chemicals. Dithizons colorimetric method and polarography are consequently not obsolete.

Isotopic dilution methods. Very interesting for levels as low as 10^{-1}–10^{-3} ppm which can be registered with great accuracy, these methods give the limit of analysis possibilities. Perhaps for practical reasons, they have not received the full development they could obtain. Determination of mercury and of manganese can be done with this method, and should be important for organo-metal-poisoning diagnosis.

Neutron activation has been used for total organic chloride determination.

Mass spectrometry. Theoretically perfect, this method uses apparatus of such complexity, that clinical application of this method is a talk of the future. N.m.r. and e.s.r. are methods of physical chemistry, rather above medico-chemical analysis.

Infrared spectroscopy. After strict purification (GPC), many pesticides can be identified by i.r. spectroscopy. Carbamates (of low toxicity if any), are determined to 0·2 ppm in carbone sulphide. Some S-phosphates can be oxidized in sulphone and dosed (Frehse).

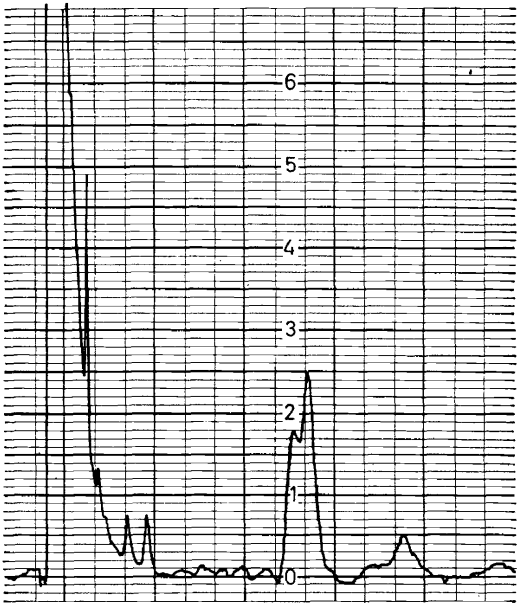

Figure 5. Electron capture detection of fat extract after GPC (elution or fluorosil) [method of Langlois *et al.*]

Ultraviolet spectroscopy. Frequently used as a routine method of analysis, one must insist on the difficulties of real identification. Best methods use reactivity of chemical hydrolysis, kinetics of hydrolysis, and ionisation by large pH changes (Bourdon)). Ultraviolet spectroscopy has not been applied so successfully on pesticides but can be used for many drugs. These methods shall be renewed during the next few years, thanks to acquirements of new apparatus for separation, where GPC could take the best and permit a pre-identification, through R_f determination.

Polarography. This excellent physical method is reliable after purification, for DDT and parathion. It has been developed for defoliants and has good prospects for application in clinical toxicology.

Gas chromatography. In the field of chromatography, progress in last ten years has been tremendous. In gas chromatography we inject some fluids and receive a chart with peaks and graphs whose detection can be at once identification and quantitative measurement.

Organochloride detection. This is an excellent application of sensitivity of electron capture detection (Guiochon). Methods for preparation of samples are well elaborated and sometimes very simple. Mills classical method cannot be used with biological samples because of fat content. Langlois method uses purification on Florisil, followed by elution with methylene

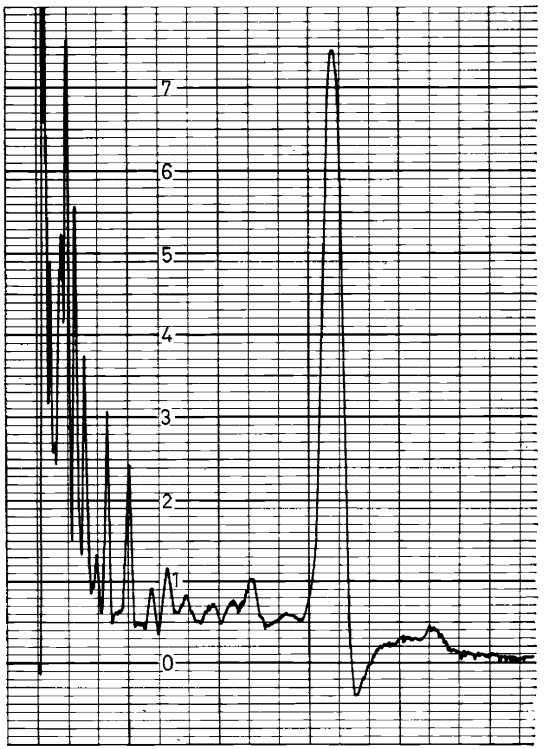

Figure 6. Electron capture detection of urine extract after GPC [method of Cueto-Biros]

chloride–ether mixture (cf. *Figure 5*). The curves obtained are excellent for biological evaluation.

Robinson–Richardson Method (*Figure 1*) for blood is very quick. It consists of direct filtration through silica gel of mixture of blood and acetone followed by hexane extraction.

C. Cueto and F. Biros use, for urine examination (*Figure 7*), a mixture of acetonitrile and urine which they extract with hexane (secondary purification by evaporation, re-dissolution).

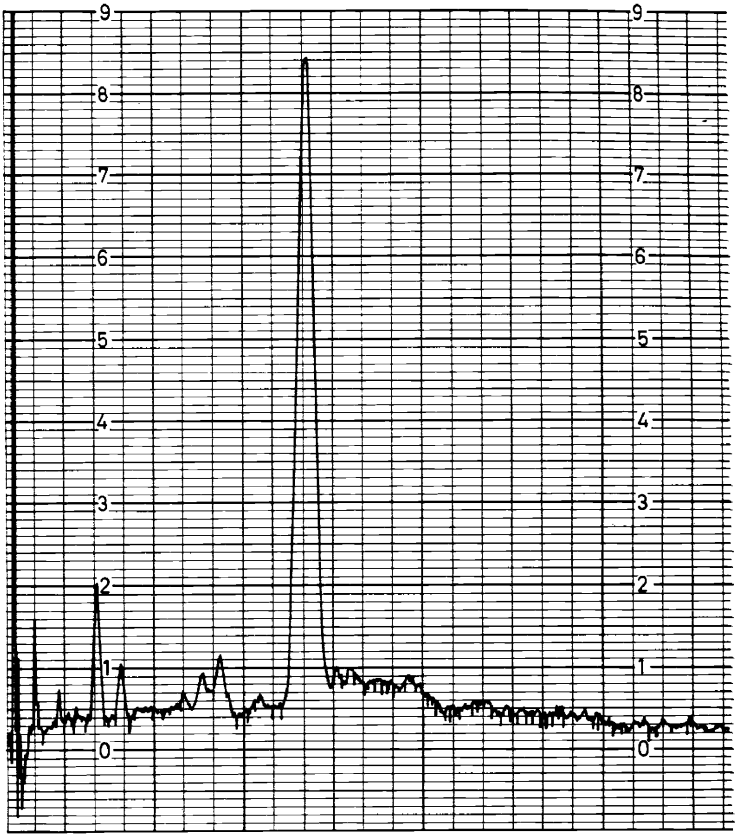

Figure 7. Electron capture detection of bone-marrow extract after GPC [method of Robinson and Richardson]

Sensitiveness of these methods is excellent but utmost attention has to be paid for careful manipulations, perfect purity of solvents and cleanliness of materials (e.g. syringes). Soft ware is not simple and determinations are to be controlled very often with standards. The choice of column packing is very difficult due to the necessity of good separation, without isomerization or destruction; polar, non-polar, polymers, proton exchanging materials are

very numerous and point to great technological achievements.

Half way between column chromatography and distillation, well controlled by sensitive detectors like electron capture detectors (Tritium or Ni 64) this method is not far from nanogram detection after it is coupled with separative GPC and pyrolytic identification.

RESULTS ON HUMAN INTOXICANTS

Despite the progress of biochemical work, interpretation of graphs is not easy. Differences between columns used by chemists lead to some misunderstanding and even very specialized analysts cannot always give a simple interpretation of results obtained by experimentation using different apparatus and different columns. Early peaks are very numerous, non-identified, and probably due to biological compounds, groups of HCH (frequently triple) interfere with biological compounds and, with columns like QF1, metabolic degradation products of DDT whose level is always high interfere with dieldrin peak which has the main toxicological importance, but remain 10–100 times lower than DDT metabolites.

As in the case of work on DDT and HCH (reported earlier) we shall take care especially of newer pesticides and Aldrine derivatives. In body fat DDT (as DDE) was found at levels 0–11 ppm. In "industrial" farmers its concentration goes up to 20 to 100 ppm; but real DDT is much lower (0·02 to 0·3 ppm). With progress in methods DDT and DDE can be found in urines at levels 10^{-3} ppm for DDT and 10^{-2} ppm for DDE but variations due to professional activity are not very large and this test cannot be used now as a survey routine-test. In blood, general levels are 0·02 ppm of DDE.

The use of HCH and isomers makes new peaks appear in chromatograms and it is not easy to identify them especially when present in amounts less than 1 ppm in fat and 10^{-3} ppm in urine.

The group Aldrin, Endrin, Dieldrin (HEOD) is now regularly found in human fat. Metabolic transformation of Aldrin in Dieldrin (Korte, Robinson) is made in liver so that dieldrin fat level is always high in experiments after absorption of aldrin or dieldrin.

The level of fat in human body stays around 0·2 ppm; but this rises to 6 ppm by insect-killers. Correlation between fat contents and day food intake is not firmly established by observation on dogs (e.g. dogs receiving 1 ppm of dieldrin have 3·4 ppm in adipose tissue and those receiving 3 ppm, 45·5 ppm).

Robinson–Richardson method using elaborate columns [sensitiveness $= 10^{-4}$ ppm) gives blood levels (in HEOD) of 1 to 4×10^{-3} ppm] (cf. *Figure 8*).

Dieldrin has been found in urine at the concentration of 10^{-3} ppm (mean normal people), rising up to 10^{-2} ppm and even to 0·5 ppm during strong exposure. This very recent work has a great practical importance showing the possibility of a survey of land workers. The control of milk levels look important in pediatric long term studies (Egan, Goulding).

In France indeed it looks rather difficult to oblige workmen to suffer repeated adipose tissues biopsies. We have done some bone-marrow punctures to improve validity of blood dosages (cf. *Figure 9*). General impression on these dosages is their numerical insufficiency as compared to tremendous

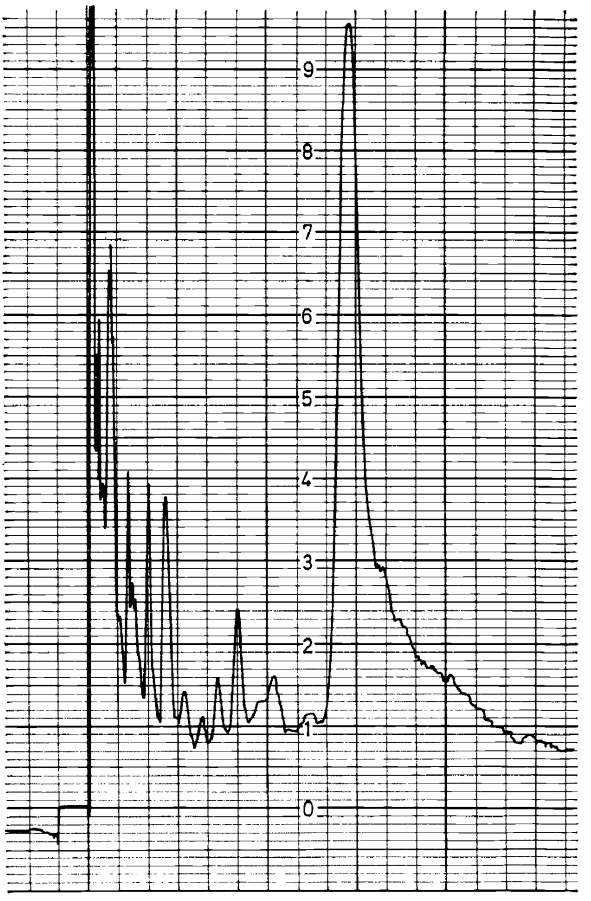

Figure 8. Electron capture detection of blood extract after organophosphorus poisoning

use of pesticides. New drugs are generally controlled by many analytical, toxicological, pharmacological and clinical experts before use and their use remains always under very strict and careful scrutiny. With pesticides on the contrary, control looks quite informal; tons of products are widely spread without much care to inhabitants and many nations have no laboratories specialized in these problems; it looks quite dangerous to enter chemical era at full speed and without brakes and we, physicians, know that a great part of modern pathology shall be human toxicology.

Organophosphates. Detection of organophosphates has always been much more difficult than organochlorides. Extraction procedures differ depending upon the chemicals themselves, to the partition coefficients in water–oil. Technical evolution is so quick that it is not easy to make a choice between the available methods.

Electronic capture after GPC (extracts with ethyl methyl ketone–hexane) appeared much less sensitive than for organophosphates, so great errors

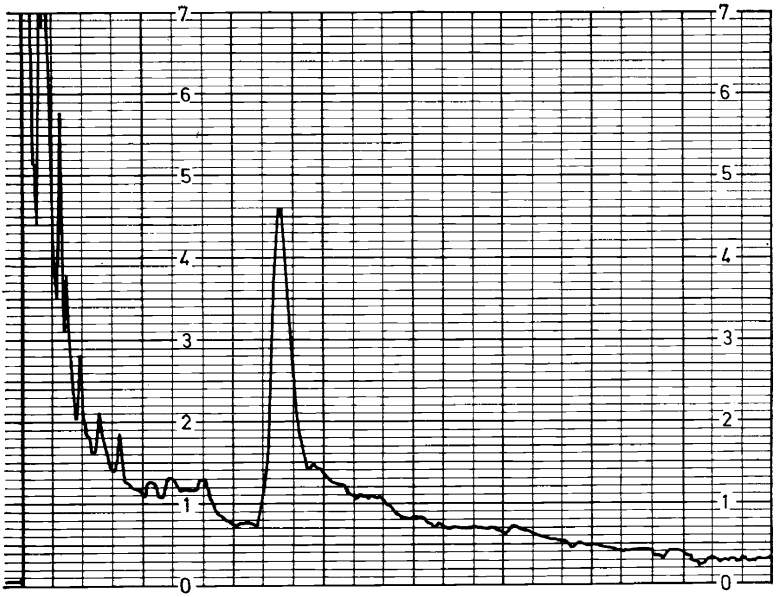

Figure 9. Electron capture detection of bone-marrow extract after GPC [method of Robinson and Richardson]

could be expected, due to constant association of both impregnations, but we observed positive responses in blood extracts during intoxication by organophosphates.

During the last few years, new detectors have been developed either by modification of flame detectors or by new detectors especially adapted to organophosphates (Giuffrida). Polarography has been used for parathion determination (Gayan).

Thin-layer chromatography. Numerous reactions have been tested during the last years. A general one is based on hydrolysis and phosphate reactions (molybdenum blue) (Coffin and Savary). Others, are more specific of thiophosphates, reacting products being sulphur compounds produced by hydrolysis (hydrogen sulphide, thioglycollic acid, phosphothioates). Others are characteristic of chlorophenol radicals (*N*-aminoantipyrine), of *p*-nitrophenols. Many reactions have no specificity and, as human biology tests, we cannot hope direct and important applications before having described new approaches of identification of sulphur compounds, and phenols (chloro, nitro and amino) in blood, urine and tissues. Use of GPC for phenols has been studied in some laboratories. Microcolorimetry of thioderivatives or polarographic determinations of purified biological extracts would be interesting in fairly short delay and could be tested after direct addition and physiological absorption of pesticides. Today we have not checked methods for clinical toxicology of new organophosphate pesticides.

Biological measures. Due to their quick biological destruction by *B. esterases*, organophosphates cannot be identified during chronic intoxication by

procedures described during acute intoxication. Because of their activity on cholinesterases, organophosphates can be studied by determination of cholinesterase activity.

A new and very interesting orientation has been given by Bruaux for controlling multiplicity of cholinesterases on agar-microelectrophoresis and observation of inhibition of some of them by small quantities of organophosphates (0·05 ppm).

Methods based on thin-layer chromatography have a sensitivity ranging between 0·1 ppm and 3 ppm. Most of them have never been tried on human biological samples and we have no proof that they would be successful. Even preparation of samples, immediate inhibition of esterases after sampling, is not described. For extraction the rather unfavourable partition coefficient methods may conduct to progressive methods like counter current enriching.

As clinical routine procedure, we trust on Michel method for good determinations of cholinesterases; during progressive poisoning this method is still the best one and must be considered as reference for surveying enzyme level variations. But clinical symptoms can be puzzling, because they can appear very late and correspond to very low levels of cholinesterase activity.

A fall under 40–50 per cent of normal scale must be considered as a a good argument for eviction of work, specially for flying-crew.

Physiological methods like electroencephalography (EEG) are not used as systematic detection tests because abnormalities are by no means specific and do not coincide with definite enzyme variations.

Association of organophosphates and organochlorides. In experimental toxicology organochlorides raise lethal dose of organophosphates. For example on rat, aldrine raises by seven the lethal dose of parathion. On many we have few records about this important biological problem and our results cannot give any answer.

Techniques of selective detection of phosphorus, sulphur and halogen compounds by GPC of pesticides are in progress (Burchfield).

Nitrophenols. Chronic intoxication with nitrophenol is detected in France by the standard method of Derrien. In fact this method is not sensitive enough and cannot be proposed for farm-work survey. New methods of gel permeated chromatography or I.R. spectroscopy or more accurate colorimetry has to be chosen (Goldmaker, Khokholkova).

Herbicides. New methods have not been applied to human toxicology concerning arsenites, chlorates, pentachlorophenol. New herbicides have a very low human acute toxicity. Therefore recordings of biological levels have not been published. We have noticed some micromethods for their determination in food and we must keep in mind that some of them (CIPC) have chlorinated aromatic radicals, so they must interfere during analysis with much more toxic compounds.

A very great inconvenience of chlorophenyl derivatives is the bad taste they give to water, the bad odour they give to atmosphere, even at very low levels, which is an important environmental hygiene problem and justifies studies for plants, water and air pollution (Henshaw).

TOXICOLOGICAL PROBLEMS POSED BY WORLD-WIDE USE OF PESTICIDES

They are very important. The discovery of organochlorides in human tissues induced physicians—and everyone—to wonder if they present a real danger. Prolonged toxicological experiments on animals have been concluded as positive for aldrine group chemicals. Biological anomalies are invoked for such small amounts as a few mg/kg per day. And a great prudence is recommended for a frequent use of insect-killing sprays.

Systematic studies on potential effects on fecondity, teratology, tumor induction, on allergenicity should be indicated, at least to physicians and Public Health Administration, before heavy using and distribution among uneducated or ignorant people.

Public Health physicians have to consider that home-products are sold without information, or with printed labels whose reading looks quite ineffective and useless. Some people are now fly- or mosquito-phobic and spray great quantities of pesticides in their house. We think that it is necessary to really take care of this problem at least by routine determination of pesticides in biological samples of statistical value.

Secondary effects of pesticides are still under study. Metabolism of some drugs is modified by organochlorides. Liver weight raises before appearance of cytologic troubles. Hormonal metabolism is disturbed. Organochlorides have an inhibitory effect on microsomal hepatic hydroxylation. And some pesticides cause disturbances of virus development in hepatic cells.

Idiosyncracy. Physicians have still no answer for the questionable agranulocytosis due to DDT or Lindane. Some polyneuritises have been described. We received one letter concerning a man who two weeks after long spraying of metasystemox suffered from acute erythrodermatitis followed by polyneuritis. Another patient, after recovering from an acute intoxication by organophosphates suffered from optic neuritis.

Toxicologic significance of atypic cholinesterases (Kalow) has not been studied during chronic poisoning.

Lindane, DDT, OP, Herbicides-allergic dermatitis are now frequent and we did not notice much work of correlation between allerginicity and cancerogenis of these products although this problem has an increasing importance.

Certainly, analytical work can neither overlap medical examination nor set it aside, but both activities should be correlated. Analysis of biological samples should be perfected and physicians have to be reminded that they can never conclude without analytical or chemical tests.

As a general conclusion I would like to mention here that a large number of papers on pesticides and a variety of opinions and chemicals make difficult a sane evaluation of this new toxicological danger. Some pesticides leave a residue in human tissues. Some disturb normal metabolisms. But we do not know if it is a real danger, and Decker wisely mentioned that the discovery of residues in adipose tissues led to reject some well-known pesticides for new ones whose toxicity was not so clear. And even among physicians, we have to deplore either extreme mistrust conducting to incrimination without proof, or neglect of new chemical dangers which cannot be

classified in a standard reference manual. We know that new pesticides can be really atoxic, or much less dangerous for man than old ones (rodenticides are the best example of such progress). On the contrary, demonstration on animals of permanent biological deviations is actually alarming. New chemicals should not take place of old ones if potential risks, balanced to activity, are not lower. Technology of application is a very important point to consider, especially for home use. Physicians and analysts have to endeavour improving their coupled contribution in this important field of health protection.

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