Ann. Ist. Super. Sanità
Vol. 21, N. 4 (1985), pp. 489-498

SCIENCE AND TECHNOLOGY IN AN INSTITUTE OF PUBLIC AND ENVIRONMENTAL HEALTH

H. COHEN, A.L. van WEZEL, B. SANGSTER, G. de GROOT and N.D. van EGMOND

National Institute of Public Health and Environmental Hygiene, Bilthoven, The Netherlands

Science and technology in a Public Health and Environmental Hygiene Institute

A survey is given of the scientific disciplines applied in the Public Health and Environmental Hygiene Institute of a middle small–sized developed country: (*a*) to study the source, transmission and the effects on human health and the impact on biotic systems in the environment of microbiological agents and chemical and physical pollutants and (*b*) to prevent or treat their effects in man. This evaluation is based on conventional epidemiological principles, which now should also be translated into modern approaches such as hazard identification, risk estimation and risk appraisal to be used as a basis for decision–making processes within the competent agencies or the ministries involved and in case of incidents, for action taken by the inspectorates of health and the environment. Functions and purposes of the Institute should be clearly defined and regularly reassessed. This will ensure timely reorganizations and reallocations as these become necessary due to the scientific developments and social changes taking place in Western societies. Modern organizational techniques, such as time accounting and allotment applied to this end, function both as steering and control mechanisms. In this way a number of tasks can be defined *viz.* a research function described in extended activity programmes to be approved annually by the ministers; production tasks (vaccine production for the national immunization programmes and pre- and neonatal screening programmes) reference and study tasks (for preventive of repressive control in the field of food hygiene, air, water, chemical, serums and vaccines, drugs, blood and blood products and some diagnostic laboratory products and procedures) and finally national and international advisory tasks. On this basis a number of problems related to health and environment can be assessed by acquiring the available scientific information and, after its integration for the purposes required, by applying it to solve the relevant issue raised by society. Three practical examples concerning vaccine production, clinical toxicology and air pollution will be given.

Introductory remarks

In medical school, during lectures in bacteriology, hygiene and preventive medicine, two principles used to be stressed. The first had to do with bacteriology and was referred to as the five postulates of Koch. The gist of it was that one may assume a relation to exist between a disease and a micro–organism, isolated from the patient, if certain conditions are fulfilled bearing on its isolation in all clinical cases of the disease, infection of animals with the cultured micro–organism and its reisolation from the infected animal. The postulates of Koch have recently been re–emphasized in the announcement that HTLV 3–virus was considered to be the cause of the AID–syndrome.

The second principle concerned the epidemiology of infectious diseases. It was recognized that each (micro) biological agent had a source, from which it was transmitted to man, and its ability to cause an infection would then depend on a number of specific and non–specific defense mechanisms of the body.

Before microbiological agents had even been recognized as causes of infectious diseases, epidemiologists interfered already succesfully with the transmission of cholera. Their role in delineating infectious diseases as separate entities is often forgotten now and should therefore be stressed whenever possible. Application of both these principles in preventive and curative medicine has, in the past, enabled medical scientists in many instances to control the spread of infectious diseases. In the last decades, knowledge of the interaction of infectious agents — bacterial, viral or parasitic — with the immune system of man has increased almost logarithmically, and has vastly deepened our understanding of the processes involved. In many instances, we can now define the essential parameters of what we call the natural history of a large number of diseases and this has diminished the interest in epidemiology, as a basic discipline in infectious diseases.

Epidemilogists have, however, in the past twenty hears, shifted their interest and have again played a pioneering role in establishing the fact that cancer is not a curse of nature, but that the different tumors in target organs in man are related to geografically determined life styles such as smoking and food habits. Their justifiable conclusion has been that chemicals whether naturally occurring or man–made do play a role as causative agents of this disease complex.

It can therefore be stated now that the basic epidemiologic approach in analysing and un–ravelling the problem of toxic chemicals including carcinogens, has been very similar to the approach used more than a century ago with regard to infectious agents. But there is more. Knowledge of the genetic structure of living organisms is expanding rapidly, and, in addition to certain constitutional diseases, its role in the final outcome of infections by microorganisms, or exposures to toxic chemicals, is now becoming clear. Once the meaning of man's gene sequences will have been fully deciphered, and his genetic code transcribed into computer memory, coming generations of medical biologists will have a reliable tool available for the analysis of strong and weak features occurring in an individual's genetic material, including his predisposition for diseases which might be triggered by exogenic factors.

Already now individual variation in susceptibility to chemicals can be predicted from observed differences in the reaction to drugs and certain other substances and it becomes clear that a cascade of genetically directed enzym systems will deactivate chemicals and protect the macro–organisms in a similar way against their toxic properties as the immunological system does against infectious agents. Gaps continue to exist, however, in our understanding of the individual diversity of both defense systems, and their relation to disease susceptibility of individuals.

Some insight has, however, been gained, during the past few years, in the interaction between chemicals or their metabolites, with the genetic structure. The recent discovery of oncogenes as components of the normal genetic structure will, in my opinion, ultimately strengthen the arsenal of tools available for the toxicologist.

My general conclusion is that it is permitted to compare chemical agents, with infectious agents in regard to the epidemiological approach, as well as their interaction with genetically controlled defense systems of the macro–organisms which, in the end, will determine the outcome of disease in individuals.

Research in a public and environmental health institute should therefore deal with both topics. It should also be mentioned here that the methods used in classical epidemiology, concerning identification of an agent, analysis of its source and transmission, should continue to play a major role in the institutional activities.

Table 1. – *Risk assessment processes of exogenic factors hazardous for the health of man or the environment and of a chemical, microbial or physical nature*

Hazard identification	Risk estimation	Risk appraisal	Risk management
Information			
Accident	Forecasting	Economic	Recognizing
Occupational	Modelling	Commercial sensitivity	Gaining control
Environmental	Epidemiolo-gical	Public perception	Solving
Screening	Toxicological	Subgroups at risk	Maintaining control
Microbiological			

based on WHO Regional Office Europe
ICP/PPE 009 (2) 19 28-11-1983

The above mentioned tasks have been reconsidered at a recent conference organized by the European Regional Office of WHO which was devoted to risk assessment and its use in the decision–making process for chemical pollutant control. A classification of risk assessment was introduced (Table 1) which distinguishes between hazard identification, risk estimation, risk appraisal and risk management. It is obvious that hazard identification and risk estimation of exogenic factors, whether of a chemical, physical or microbiological nature, are based on clinical, epidemiological, toxicological and microbiological information on the natural history of the disease process involved. Risk appraisal refers both to the analysis of the perception of the disease existing in the community and to scientifically established facts.

It should therefore be carried out in close cooperation between scientists in the above mentioned fields and policy making agencies in the responsible ministry or agency. On the basis of its outcome the politically responsible ministers can then devise the strategy to be established for risk management. Recently a model was worked out by the Minister of Environmental Hygiene in The Netherlands, Pieter Winsemius, in which four phases in this process are distinguished (Fig. 1).

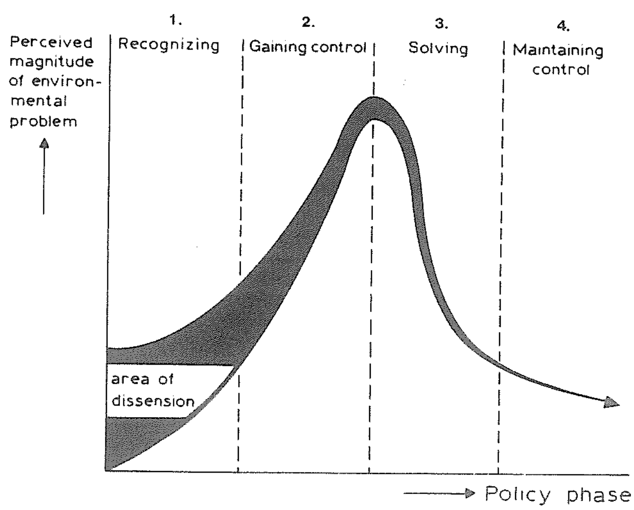
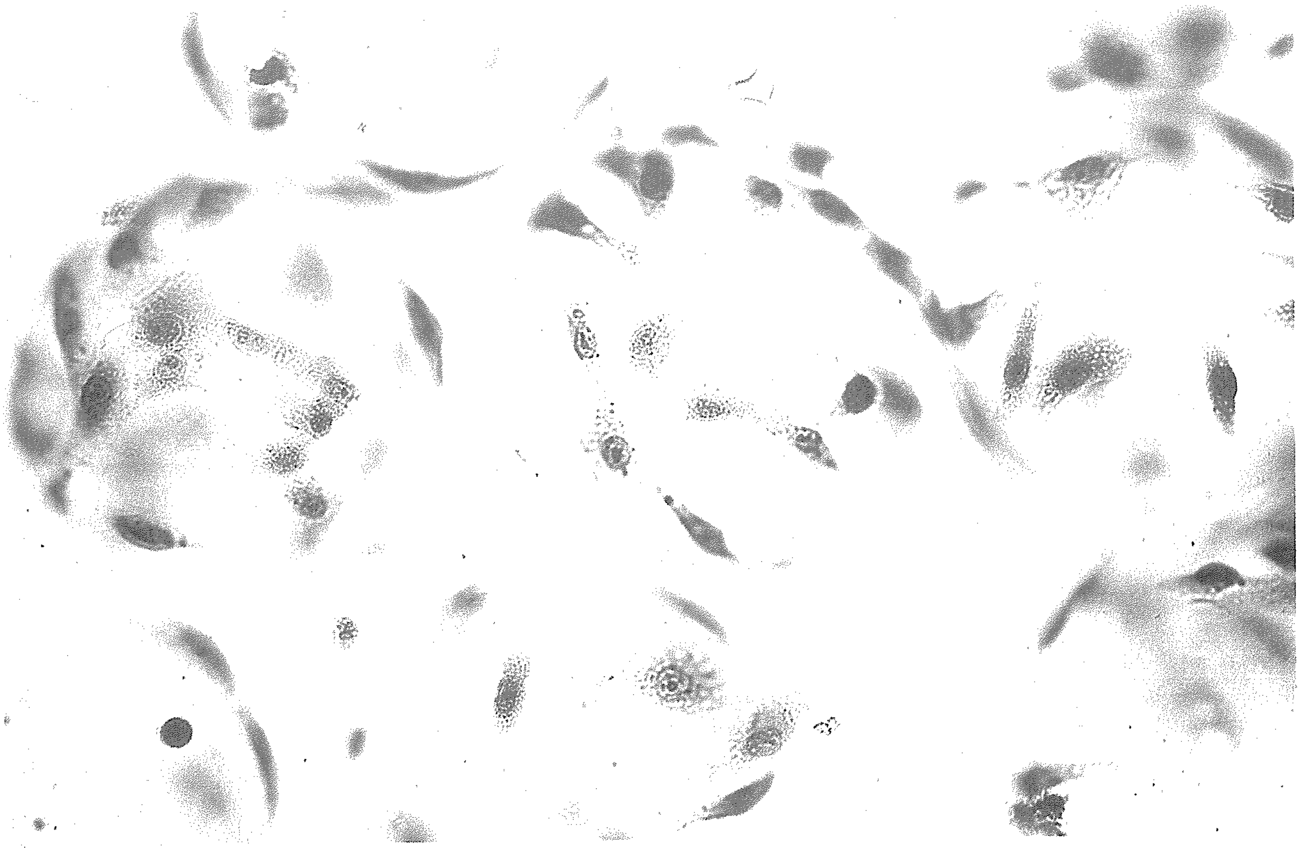


Fig. 1. – The environmental policy life cycle



a)

b)

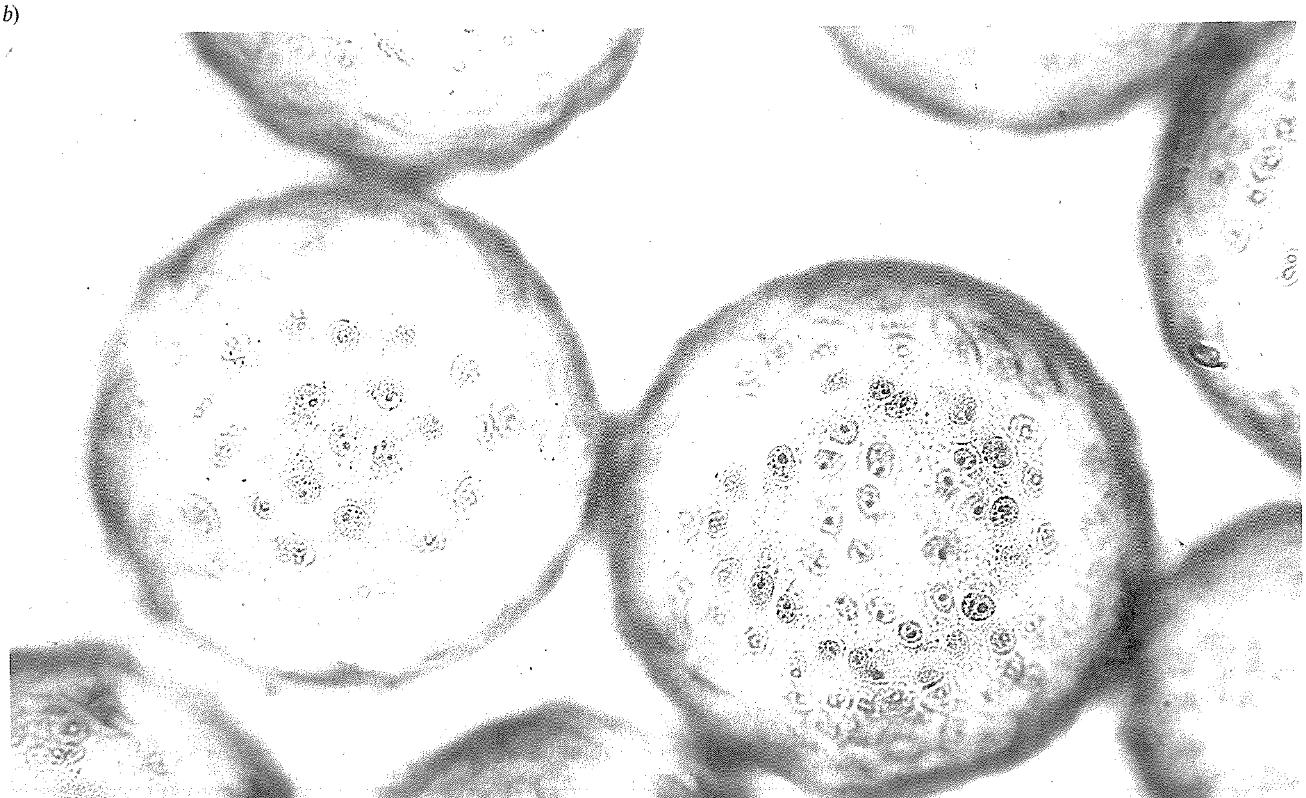


Fig. 2. – Microcarrier cultures of vero cells: *a*) 24 hours after inoculation; *b*) 96 hours after inoculation

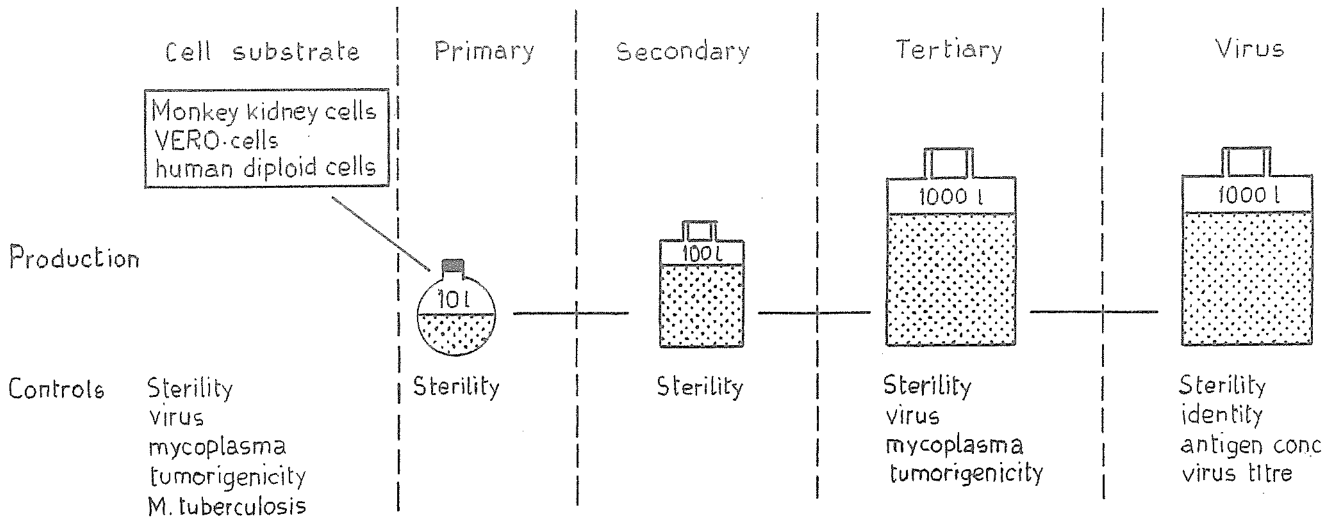


Fig. 3. – Large scale cell/virus cultivation system for production of inactivated poliovaccine

It is clear that in the "recognizing" and "gaining control" phases the uncertainty and dissension area are considerable. Here the well organized information system of a public and environmental health laboratory can play a decisive role.

In my opinion scientists in such an institute should have no direct responsibility for the decisions of policy making agencies and should limit their role to that of advisers and investigators by setting up and guarding reference systems. A public and environmental institute should therefore work for the central government and its agencies, such as the chief inspectorates of health and environment. The position of the institute, amid a rapid development of the scientific disciplines concerned and social changes in our society should be clearly defined and regularly reassessed, in order to implement necessary reorganisations and reallocations in time. This can be done by applying modern organisational techniques such as an annually extended active programme, controlled by time management which functions both as a steering and control mechanism.

National Institute of Public and Environmental Health in the Netherlands

The Institute comprises three divisions:

- Microbiology and Immunology;
- Toxicology and Pharmacology;
- Chemistry and Physics.

An example of research in each division is given.

Example I – Division Microbiology and Immunology

Production and application of inactivated poliomyelitis vaccine. – For many years a broader application of inactivated polio vaccine has been hampered by high production costs. The development of the microcarrier culture at our Institute has made large scale production of this vaccine economically feasible at an industrial scale. In microcarrier culture the cells needed as substrate for virus cultivation are grown on small particles suspended in the culture medium (Fig. 2). In this way also normal diploid cells which only grow *in vitro* after attachment to a suitable surface, may be cultivated in large bioreactors of 100 to 1000 l and more (Fig. 3). With modern fermentation apparatus, optimal culture conditions can be maintained in the bioreactors during the cell and virus cultivation process which garantees a consistent high cell and virus yield. The crude virus suspension is concentrated, purified and inactivated and may be mixed with the bacterial vaccines against diphteria, tetanus and pertussis to a combined DTP–polio vaccine as outlined in Fig. 4. The concentration of the antigen required for induction of neutralizing antibodies, the so called D–antigen, can be accurately measured in the vaccine by serological tests such as the radio assay and enzyme linked immunosorbent assay. On basis of this D–antigen determination vaccines of various antigen concentrations have been prepared in order to define the required amount of antigen for induction of neutralizing antibodies after one dose. In collaboration with Dr. Jonas Salk and colleagues in France, Finland, Sweden and various other countries, a number of clinical studies have been carried out with these vaccines.

From these studies it may be concluded that almost 100% of the children will have developed neutralizing antibodies against the three poliovirus types after one immunization if the trivalent vaccine contains 40, 8 and 32 D–antigen units type 1, 2 and 3 respectively (Fig. 5). Upon a second immunization all children have high neutralizing antibody titres one month later. In addition it has been shown that the

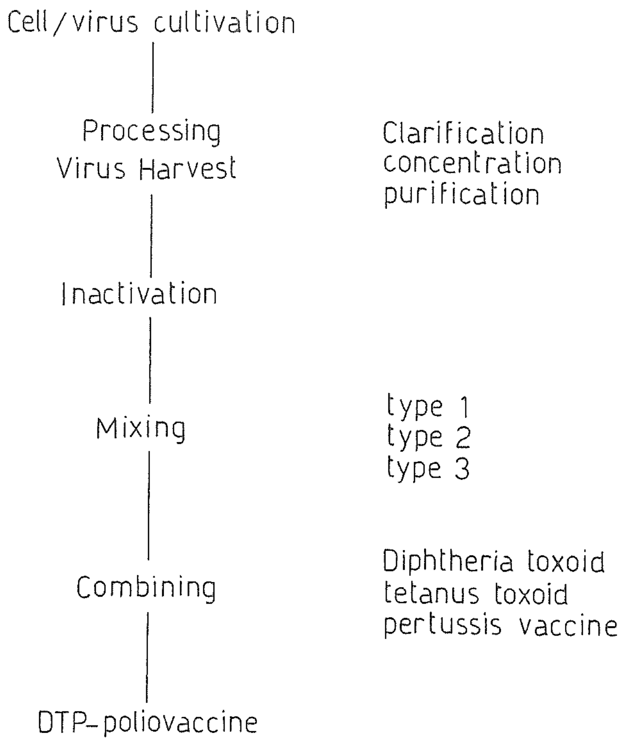


Fig. 4. – Large scale production of inactivated poliomyelitis vaccine

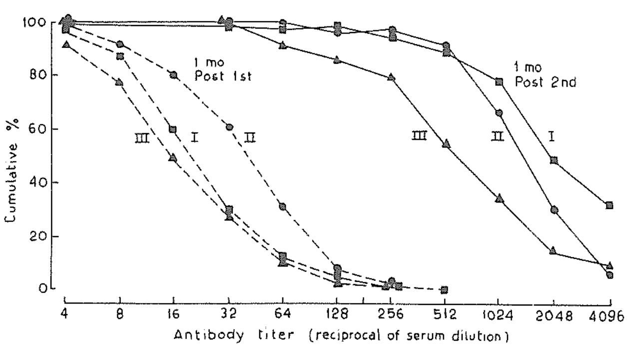


Fig. 5. – Type I, II and III antibody levels after the first and second dose of inactivated polio vaccine given at an interval of 6 months (40-8-32 D-antigen units/dose for type I, II and III, respectively)

interval between the first and second dose should be preferably 4 to 6 months in order to achieve the highest immune response (Fig. 6).

On the basis of these and clinical studies with a more potent DTP–vaccine a new DTP–polio vaccine for use in a two dose schedule has been formulated (Table 2). This vaccine is now applied by mobile local medical teams in regular vaccination in Senegal and Upper Volta according to the following schedule: children at the age of 3 to 8 months are immunized with the first dose of DTP–polio and six months later at the age of 9 to 14 months with the second dose. Simultaneously with the first dose of DTP–polio children are immunized with BCG and with the second dose with a combination of live measles and yellow fever vaccine (Table 3).

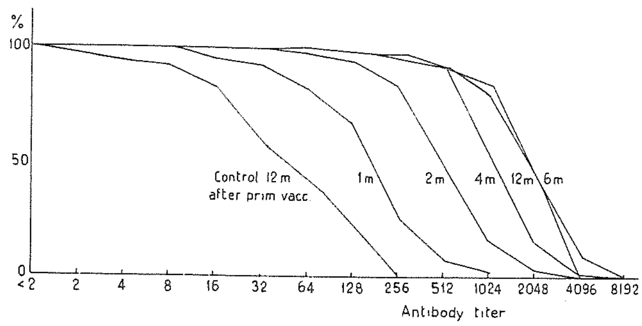


Fig. 6. – Cumulative percent distribution of type 1 poliovirus antibody titers one month after booster vaccination given at various intervals after primary vaccination. Vaccine 80 D–antigen Units

Table 2. – *Composition of DTP–polio for application in a two dose immunization schedule*

Diphtheria toxoid	15 Lf
Tetanus toxoid	10 Lf
Pertussis	16 OU
Polio type 1	40 DU
type 2	8 DU
type 3	32 DU
$ALPO_4$	3 mg

Table 3. – *Simplified immunization schedule for EPI programmes*

Age	*Vaccine*
3 - 8 months	DTP–polio vaccine BCG
9 - 14 months	DTP–polio vaccine Measles Yellow fever

Serological evaluation of both studies indicate that in this way children may be protected against the seven infectious diseases of the "Expanded Programme on Immunization" of WHO just by two visits and four injections. Meanwhile in Israel and India field studies have been carried out with three doses of DTP–polio with lower concentrations of trivalent polio vaccine. Also in these studies high seroconversion rates have been obtained. However, the advantages of such a simplified two dose immunization schedule are obvious. The administration costs of the vaccine will be lower and at the same time higher immunization coverages will be reached as children have only to be seen twice for a complete immunization. These two points certainly compensate the somewhat higher production costs of the inactivated polio vaccine. In addition it may be expected that through the replacement of monkey kidney cells by non–tumorigenic cell lines such as VERO cells for virus multiplication, a further reduction in production costs may occur. Recently the VERO cell vaccine has been licensed in France. It may be expected that these cells will soon be generally accepted which will make an unlimited production of the vaccine possible.

Example II – Division Toxicology and Pharmacology

In 1980 Lekkerkerk, a small town in the county Zuid Holland, appeared to be built upon a dumpsite where chemical waste had been disposed illegally. Since then the general public has realised that concern about environmental pollution is not only confined to environmental activists but also to themselves. In particular, the awareness of the possible relation between adverse health effects and polluting substances has dramatically increased. Particularly in the fairly large number of areas where quarters were discovered to be situated upon chemical waste, people have asked their general practitioners to investigate whether their complaints and symptoms were caused by the chemicals involved. It also has occurred that people without medical complaints who assumed that they were living in a polluted area requested to be examined for the presence of "pollutants" in their bodies.

Since 1980 the department of medical toxicology of the Dutch national poison control center of the Institute has been involved in all known cases of pollution where man could be exposed to the pollutants. Medical surveys as well as screenings were performed. Apart from providing medical care on a collective basis in some cases, in all other cases the general practitioners were advised in the diagnosis and treatment of individual patients. If necessary, individuals could be referred by their general practitioners to the department, to be examined by one of the three internists specialised in medical toxicology. Thus, quite some experience how to approach medical problems in relation to environmental pollution has been acquired.

In order to be able to perform these examinations, research has been performed. This consisted, among others, in collecting reference values for the concentrations in blood and plasma of many substances that could be involved in future cases. Assisting medical doctors in new fields of medicine and providing the necessary scientific basis is one of the tasks of the National Institute of Public Health and Environmental Hygiene.

Quite recently, one of the coworkers of the Department of Medical Toxicology was called by an internist, who asked the following question: "Do women with an increased copper body burden due to environmental pollution have to be advised against future pregnancy because of danger for the child?". A rather unusual question.

What has happened? Several persons living in adjacent houses supposed that they were living upon a dumpsite. They went to their general practitioner and asked him to be examined for the presence of "toxic substances". Because he had recently received a brochure of a laboratory that offered its services to perform analyses of bloodsamples for metals, he decided to determine the concentration of several metals. Samples were obtained and the inhabitants went home waiting for the results of the tests. Everybody was surprised when in several inhabitants, all women, plasma copper concentrations appeared to be "too high". It is a common practice in medicine to repeat tests of which the results are abnormal. When again plasma copper concentrations in these women were increased, the assumption that they were living in a polluted area was confirmed. Anxiety increased and questions were asked. Definitely copper was one of the pollutants!

The women in whom abnormalities were established, were referred to the internist who consulted the department. Before, he had determined the plasma copper concentrations once more and had repeated the analyses when again concentrations appeared to be increased.

The above–mentioned determination of reference values was the basis of the answer. In 56 healthy volunteers (26 males and 30 females, age 19-29) first the medical status was meticulously established. Among many substances plasma copper concentrations were determined. In several women these concentrations were higher than values obtained from the literature. Because the medical status was known there was no reason for immediate concern. The importance of obtaining this knowledge before establishing reference values is stressed by the fact that this provided the key to the solution of the problem.

Females were subdivided according to the use of oral contraceptives (o.c.) (Fig. 7). The concentrations in o.c. using women were significantly higher. One female not using o.c. nevertheless had a high concentration. She had used the so called "morning after pill" several days before the blood sample was taken.

Enzyme induction by o.c. causing an increased synthesis of the copper transporting protein, ceruloplasmin, was suspected. The results obtained after subdividing the plasma ceruloplasmin concentrations measured in the same samples (H. H. Kamp, University Hospital, Utrecht), confirmed this hypothesis (Fig. 8). Mean plasma copper and ceruloplasmin concentrations respectively of males and females not using o.c. did not differ (Table 4). The mean plasma copper and ceruloplasmin concentrations of females using o.c. is significantly higher than those in males and those in not o.c. using females ($p < 0.001$, $0.01 > p > 0.001$ respectively using Students' t– test).

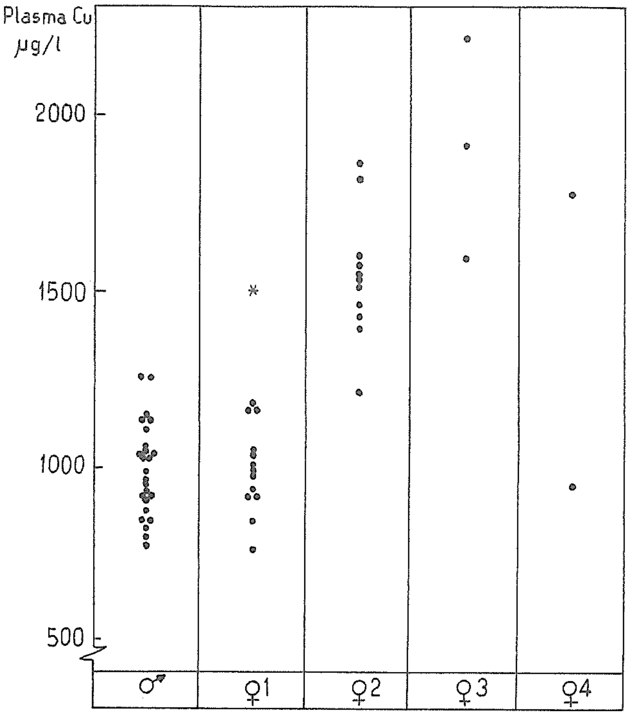


Fig. 7. – Plasma copper concentration in 56 healthy volunteers (age 19–29) subdivided according to sex and the use of oral contraceptives. 1 = no oral contraceptives (note* recent use of "morning after pill"), 2 = Microgynon 30®, 3 = Ministat®, 4 = Stediril–d 150/30® and Trinordiol®

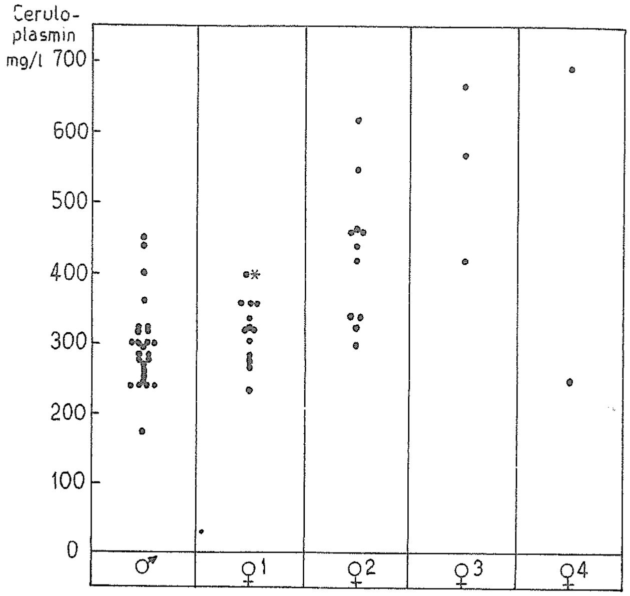


Fig. 8. – Plasma ceruplasmin concentration in 56 healthy volunteers (age 19-29) subdivided according to sex and the use of oral contraceptives. 1 = no oral contraceptives (note* recent use of "morning after pill"), 2 = Microgynon 30®, 3 = Ministat®, 4 = Stediril–d 150/30® and Trinordiol®

These observations in the context of obtaining reliable reference data stress the need for knowing exactly the medical status of the individuals whose blood, urine or other specimens are used for establishing reference or so called control values. Not following this strategy in this case might have led to the conclusion that the mean plasma copper concentration in men and in women is different because o.c. as a possible factor in increasing plasma copper concentration might have been overlooked.

On the basis of the described results the internist requesting advice was asked whether the women with the increased plasma copper concentrations were using o.c. When this was confirmed it was concluded that the copper concentration was induced by taking o.c. and not by living in a polluted area.

Example III. – Division Chemistry and Physics

Environmental monitoring systems. – The effects of air pollution, nowadays manifesting themselves in the acidification of soil and water as well as in the

Table 4. – *Mean plasma copper and ceruloplasmin concentrations in 56 healthy volunteers (age 19-29). Subdivided according to sex and the use of oral contraceptives*

	Number	Plasma copper (M ± s.d.)	Plasma ceruloplasmin (M ± s.d.)		
♂	26	992 ± 132 µg/l	297 ± 62 mg/l		
♀1	13	993 ± 127 µg/l	319 ± 46 mg/l	no oral contraceptives	
♀2	11	1541 ± 183 µg/l	429 ± 99 mg/l	Microgynon 30®	
				levonorgestrel	150 µg
				ethinylestradiol	30 µg
♀3	3			Ministat®	
				lynestrenol	750 µg
				ethinylestradiol	35,5 µg
♀4	2			Stediril-d 150/30®	
				levonorgestrel	150 µg
				ethinylestradiol	30 µg
				Trinordiol®	
				levonorgestrel	50; 75; 125 µg
				ethinylestradiol	30; 4; 3 µg

dieback of forests, have emphasized the need for integrated studies which consider the transport of pollutants along their pathways throughout the environment. In monitoring the state of the environment a consistent integration of the individual programmes for soil, water air and biosphere is pursued. To support such studies, in particular to supply data for validation of integrated environmental models, existing monitoring programmes were restructured in order to comply with the need for mutually compatible data from which pollutant fluxes can be derived. The resulting monitoring system is based on the work of 16 stations located throughout the Netherlands, and comprises sampling and analysis for pollutants in air, rainwater, soil and groundwater; to study the effects on vegetation, a biomonitoring programme is also performed at these stations. A feasibility study has been started for mutagenicity monitoring. Further ecological monitoring such as lichen growth on trees, and both short–and long–term epidemiological studies are undertaken.

The monitoring of air pollutants includes indicative components, such as SO_2, NO_X, O_3 and CO, total suspend particulates, submicron aerolsols, heavy metals and organic compounds. In addition to the 16 integrated stations, the air pollution monitoring programme is supported by about 60 stations for the automated measurement of indicative components. The network is a subset of the original air pollution network of 223 stations, which had been operational since 1975. The design of the new network is based on statistical analysis of data which were obtained from the original network.

The network is intended to provide data on ambient air pollutant concentrations for comparison with public health standards, trends in concentration to evaluate the effectiveness of abatement strategies, and estimates of the contributions from both inland and foreign source areas (and source categories), to the measured concentrations and depositions.

To support governmental policies and abatement strategies, strong emphasis is laid on the development, implementation and application of simultation models, on which, in combination with the measured air pollutant concentration and emission data, scenario studies can be based.

The following examples illustrate the application of information from network and model studies to policy making:

1) Based on the application of both statistical receptor–oriented models and numerical simulation models for the 400 x 400 km^2 mesoscale surroundings of the Netherlands, the contribution of foreign source areas to the average SO_2–concentration for the whole country was estimated at 70%. Within

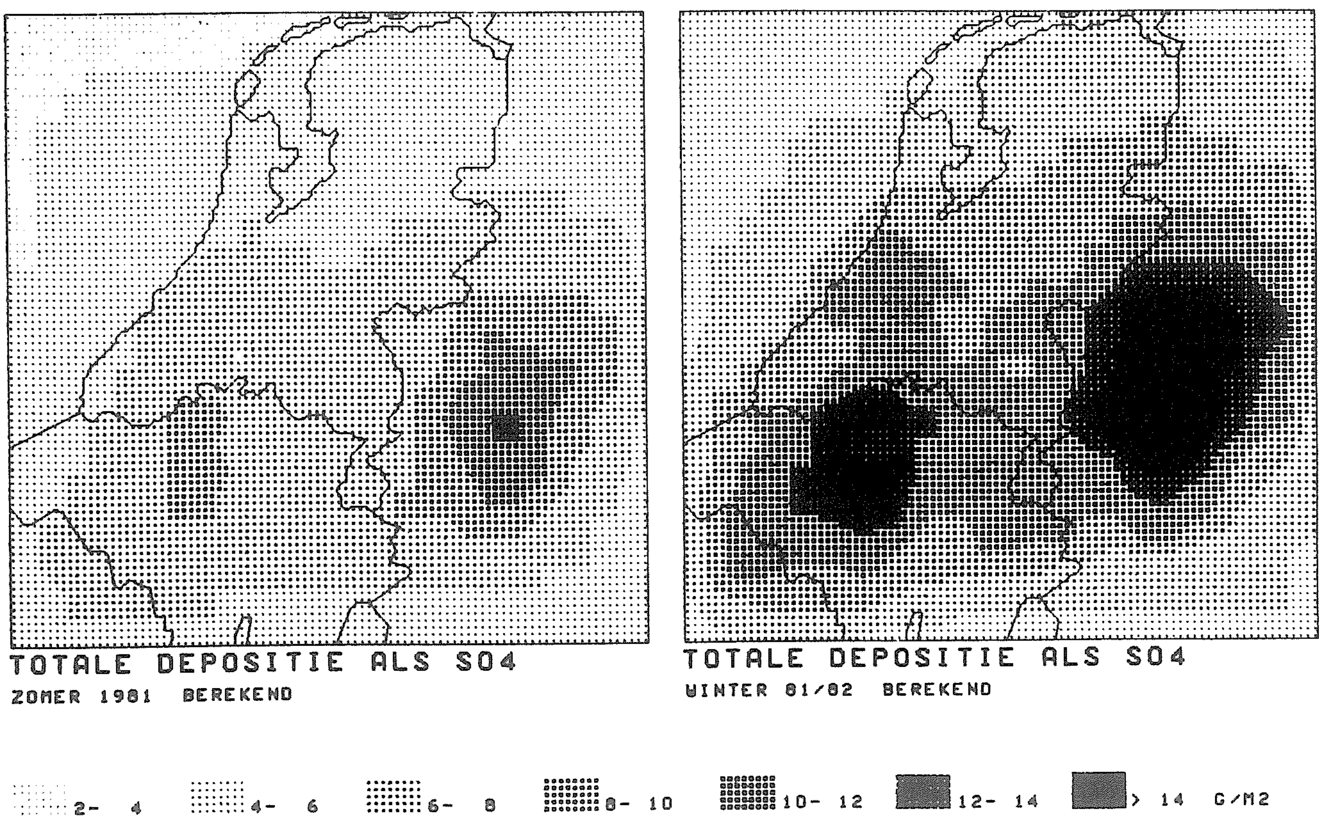


Fig. 9. – Computed total sulphur–deposition in summer and winter in the Netherlands

the major Dutch industrial area (Rijnmond–Rotterdam) the foreign contribution was still 35-50 %. For NO_2 foreign contributions to country–average concentrations were estimated at 60%.

2) Acid deposition was estimated to originate for about 3000 eq H/ha/year from SO_x, and 1200 eq H from NO_x. The contribution of the combined Dutch sources to total S–deposition in the Netherlands was estimated at 23%. As an example the computed total S–depositon field is given in Figure 9.

3) Exceedances of SO_2–standards were reported for the above mentioned (Dutch) major industrial area, and for the border areas with Germany and Belgium, due to transport from nearby source areas; a 98–percentile map is given in Figure 10.

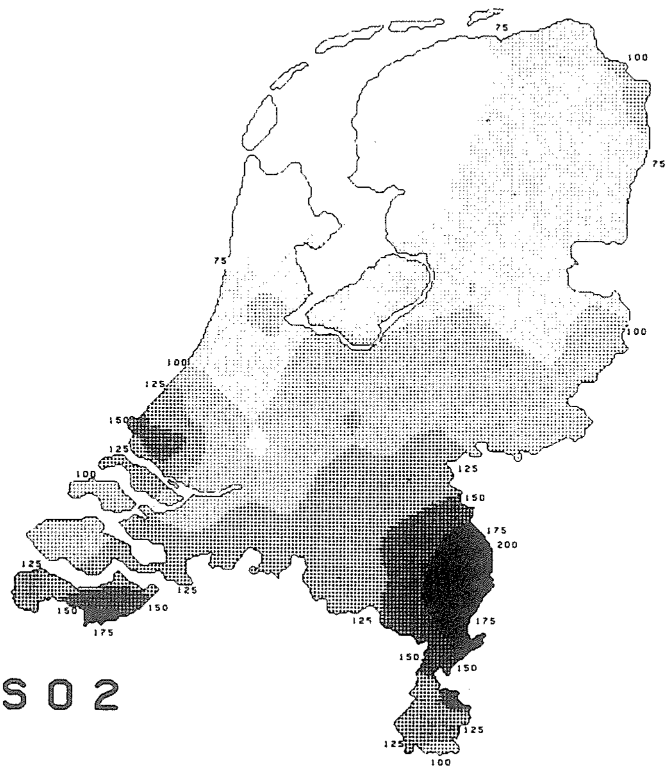


Fig. 10. – 98–percentile. Year: April 1981 – April 1982. Daily average concentration in µg/m³

4) Trends in SO_2, NO_x and O_3 pollution reflect trends in economical activity, on which meteorological variability is superimposed. Application of models which account for this variability allows estimates of emission trends, as well as evaluation of the effectiveness of policy measures.

5) Pollutant concentrations during episodes are minimized by increasing the use of low–sulphur natural gas in power plants. Following online transmission of SO_2–measurement data to the national coordinating centre for electricity production, natural gas is allocated to power plants in zones with high SO_2–concentrations, thus avoiding excessive SO_2–levels.

The biomonitoring programme, which has been run in cooperation with the Research Institute for Plant Protection, so far has revealed, among others, significant spatial and temporal correlations between ambient O_3-concentrations and leaf injury in tobacco (indicator) plants (Fig. 11). This ozone effect, which is also suspected to play an important role in forest dieback, has shown a substantial increase over the last three years.

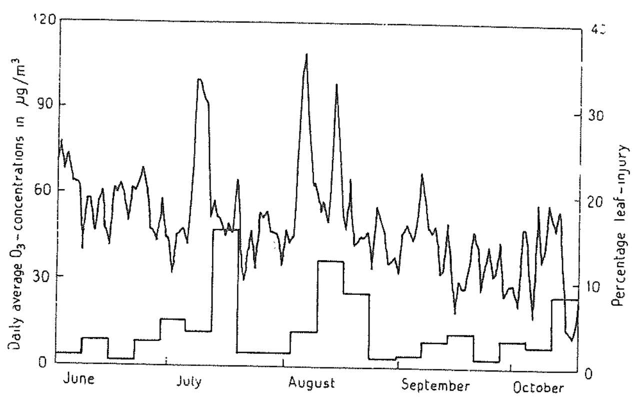


Fig. 11. – Daily average O_3–concentrations and weekly percentage leaf–injury on tobacco–plants (summer 1981)