Methods for Assessing the Effects of Mixtures of Chemicals
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Estimation of Human Exposure to Mixtures of Chemicals: Methods of Clinical Surveillance

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ABSTRACT

The effects of mixtures of chemicals are most often reported to consist of psychological and neurological disorders, toxic liver disease, nephropathy, respiratory diseases, etc. As the factors usually act at low intensity one could expect mild or even subtle changes in the different organ and system functions. This is the reason to suggest using a complex of sensitive methods and tests that allow an estimation of minor functional disturbances in separate organs. For the investigation of the nervous system a battery of psychological tests such as the Wechsler Adult Intelligence Scale, Benton Visual Retention Test, Story Recall Task, and Taylor Manifest Anxiety Scale is suggested, as well as electroencephalography (EEG) and electroneuromyography (ENM). The liver functions under examination should include enzyme activity measurements (liver circulatory enzymes, e.g. glutamyltranspeptidase, ornithine carbamyltransferase, leucine aminopeptidase, sorbitol dehydrogenase), pigment, protein, and excretory functions, lipid metabolism, and detoxifying functions connected with the activity of mixed function oxidases. The most frequently used tests for nephrotoxicity are blood urea nitrogen and plasma creatinine. The investigation of the following biochemical indices of nephrotoxicity is recommended: (a) measurement of urinary total proteins; (b) urinary β-*N*-acetyl-*D*-glucosaminidase; and (c) urinary β_2-microglobulin. The types of studies relevant to the objectives of a male reproduction risk assessment should include: (a) semen analysis for volume, sperm concentration, total sperm per ejaculate, and sperm motility (at 37°C); (b) abnormalities of spermatozoal morphology; (c) blood hormone levels; (d) data on testicular size and consistency; and (e) reproductive history. In the assessment of an effect on the female reproduction function, a study of menstrual cycle disorders, the ability to conceive, and pregnancy outcomes is recommended. Also, biological monitoring for genotoxicity by cytogenetic analysis of structural chromosome aberrations and sister chromatid exchange (SCE) is advisable.

1 INTRODUCTION

Irrespective of the fact that man is frequently exposed to the effect of mixtures of chemicals in both working and living environments, there are no developed criteria and adequate methods for estimating combined effects. When the effect concerns populations, the presence or absence of the effect can be demonstrated by the respective statistical methods. However, for assessing the reaction of an individual it will be more complicated to relate a definite illness to the combined effect of chemicals.

The character of the combined effect depends on the dose, duration, and ratio of the individual components. Most frequently, the specific effect is dominated by the leading component of the mixture.

When there is an effect of combined factors that is of small intensity and persists for a long time, usually the effects are unspecific and are manifested as illness of general character (Fridland, 1966). The differentiated aetiological diagnosis in these cases is very difficult.

In epidemiological studies concerning combined effects the most frequently reported were toxic encephalopathy and neuropathy, toxic liver disease, toxic nephropathy, toxic myocardial and vascular disturbances, diseases of the blood, respiratory disturbances, allergic states, and skin alterations. The reproductive function for both men and women might also be disturbed.

2 EFFECT ON THE NERVOUS SYSTEM

The chemical toxins might provoke functional and morphological disturbances of the central, peripheral, or vegetative nervous systems. Some substances have neurotoxic effects and cause characteristic and specific changes (e.g. manganese, carbon disulphide, mercury, lead, organophosphorus compounds). Others act as narcotics (e.g. organic solvents). Most vulnerable is the cortex, but after continuous exposure other structures are also affected.

In the Soviet literature, very often a description of early manifestations of combined effects on the nervous system defined as asthenovegetative syndrome has been reported (Krasniuk *et al.*, 1981). In its initial stage it is a vegetative vascular dystonia with the following phenomena: emotional instability, disturbance during sleep, unsteady pulse, and hyperfunction of the thyroid gland; next there is unusual fatigue, drowsiness, and neurotic disturbances in the functions of different systems. In experimental conditions this is an infringement on conditional-reflectory activity (Pavlenko, 1975). The organic injuries could be manifested clinically as encephalopathy, neuropathies, or psychotic disturbances. It is already known that the functional changes of the nervous system (deviations in the behaviour and neuropsychic sphere) may be an early sign of toxic effect before the manifestation of biochemical, haematological, clinical, and other changes.

With substances with different mechanisms of action (carbon disulphide,

organophosphorus pesticides, alcohol, formaldehyde, lead, mercury, carbon monoxide, polybrominated biphenyl), deviations in behaviour are observed such as delayed time of reaction, decreased capacity of perception, lack of good visual–motor coordination, memory difficulties as well as neurological symptoms (Valciukas *et al.*, 1978; Xintaras *et al.*, 1974). When using a behavioural test it should not be forgotten that the central nervous system has great possibilities for adaptation and compensation. Therefore it is necessary to apply a large battery of neurophysiological and psychophysiological methods. Methods for studying the effect of toxic substances on the nervous system are given in detail in Mitchell (1982).

2.1 Behavioural Changes

Behavioural effects are usually manifestations of the non-specific integral responses of the organism to the toxic substances, but in some cases they may be specific (Muchtarova, 1973). In some instances the concomitant behavioural changes may be unremarkable or they may be early and direct, ranging from interference with sensory and motor integration to profound intellectual deficits and emotional disturbances.

Extensive studies have been performed by Metcalf and Holmes (1969). The psychological test battery is used: the Wechsler Adult Intelligence Scale (WAIS), Benton Visual Retention Test, and a Story Recall Task. The Story Recall Task is a specific memory task which involves drawing from memory progressively complex geometric designs to test hand–eye coordination, perceptual accuracy, and memory. Results indicate that the dysfunctions most clearly seen in the exposed group are disturbed memory and difficulty in maintaining alertness and appropriate focusing of attention.

Interviews are used as an independent check on some of the results of psychological testing and for the special information that can be gathered only in this way.

Anxiety is measured by the Taylor Manifest Anxiety Scale (MAS), which consists of 50 true–false questions derived from the Minnesota Multiphasic Personality Inventory items. Aspects of anxiety covered by the items include tension, inability to concentrate, fear, restlessness, insomnia, gastrointestinal complaints, and increased sweating and heart rate.

Depressive symptoms are assessed by the Beck Depression Inventory, which consists of 21 multiple-choice items read by the subject and examiner. Items on the Depression Inventory pertain to sadness, self-depreciation, guilt, appetite, and loss of libido (Levin *et al.*, 1976).

2.2 Electroencephalography

EEG investigations are used for defining characteristics of central nervous system (CNS) disturbances (Metcalf and Holmes, 1969). There is no increase in

the incidence of hard EEG abnormalities such as spike activity or focal slowing. The usual finding in the EEG study is a high incidence of low- to medium-voltage slow activity in the theta range. More severe EEG disturbances are seen after active exposure.

Computer analysis using recently developed methods would yield specific information permitting the rapid identification of changes and permit the EEG to be used as a sensitive early index of CNS impairment.

2.3 Neuromuscular Function and Electroneuromyography

Electroneuromyography (ENM) appears to be an objective and sensitive test of neuromuscular function (Roberts, 1976; Roberts and Wilson, 1973).

Jager (1976) made an extensive review of 218 papers to examine the problem of neuropathy connected with exposure, and the use of ENM as a screening method for early detection of health impairments. He also reports his own results. The author summarizes the changes found as follows: 'A decrease in the voltage of the action potential of the muscle. In non-exposed controls most values would be between 11 and 12 mV; in the exposed workers the range would be wider and values could occasionally be as low as 4 mV. A change in the pattern of the recorded electromyogram,indicating that not all muscle fibres react at the same time to the supramaximal stimulation. A decrease in the conduction velocity of the nerve fibres.'

Jager's data on organophosphate workers indicate that the changes are reversible, but his literature review shows that in the more advanced stages which are accompanied by clinical signs and symptoms, this may not always be so. Reversibility possibly depends upon both the magnitude and duration of exposure.

The slow recovery and the changes found are in accordance with a diagnosis of subclinical neuropathy. The method of ENM examination is described by Jager *et al.* (1970) and by Roberts and Trollope (1979). More research is necessary in this field.

A new development in the study of the nervous system is position emission tomography. It is thought to be very valuable because it possibly enables reception of information on brain metabolism in people (Savory and Wills, 1981). Cerebrospinal fluid analysis provides a little information on the toxic impairment of the brain. The authors think that emission tomography could be used to study *in vivo* abnormality of glucose metabolism and energy production and neurotransmission.

3 LIVER FUNCTION

The liver plays an important role in the maintenance of the homeostasis of the organism.

Numerous methods are used in clinical practice for evaluation of the different functions of the liver. Because of its extremely large compensatory possibilities it is not always easy to prove a toxic effect in its early stage.

The enzyme activity measurements give many possibilities for diagnosis. The liver circulatory enzymes (e.g. glutamyltranspeptidase, ornithine carbamyltransferase, leucine aminopeptidase, and sorbitol dehydrogenase) have an increased activity in the serum following exposure to poisons.

The pigment function of the liver is also affected. Increased bilirubin in the serum and urobilinogen in the urine are indicators of such an impairment.

The protein liver function is very often affected, especially in heavy intoxications, and is manifested in disturbances of the correlations between some protein fractions of the serum or in the level of total serum protein.

The excretory capabilities of the liver in man are evaluated by the loading of substances which are excreted through the bile such as sulphobromphthalein, indocyanine green, bromthymol blue, etc. The clearance of the dyes in serum is traced. Disturbances in this function might be registered in the combined effect of chemicals of low exposure because of the restricted compensatory possibilities of this function.

Lipid metabolism is also affected in combined toxic effects. An increase of lipoproteins, especially of cholesterol, is very often described as something specific for toxic hepatitis.

The changes in the activity of oxidases with mixed functions reflect the detoxifying function of the liver. Microsomal enzyme induction and inhibition are non-specific integral responses of the organism to the chemicals entering the body in working conditions and environment, as well as drug and alcohol consumption. It is considered 'particularly useful to evaluate the effects of combined exposure' (Maroni *et al.*, 1981).

It is already known that many environmental chemicals stimulate microsomal enzyme activity. Such chemicals include the following: halogenated hydrocarbon insecticides (chlordane, DDT, methoxychlor, aldrin, endrin, dieldrin, heptachlor, benzene hexachloride), polycyclic aromatic hydrocarbons (benzopyrene, benzanthracene), herbicides, some dyes, solvents (benzene, toluene), ethanol, nicotine, chlorinated dioxins, polychlorinated and polybrominated biphenyls, aniline, tetraethyl lead, and epoxy compounds. Other substances such as organophosphates, carbon tetrachloride, carbon dioxide, carbon disulphide, ozone, cadmium, lead, and mercury inhibit microsomal enzyme activity (Conney and Burns, 1972).

3.1 Measurement of Enzyme Induction

Clinical methods have already been introduced for testing the induction of drug metabolizing enzymes (Popov, 1983; Siest *et al.*, 1981). The basic enzymes (Siest *et al.*, 1981) that are induced are located in the endoplasmic reticulum and are:

(a) mixed function oxidases (monooxygenase system), MFOs, the most important component being cytochrome P-450; (b) epoxide hydrolase (EH: EC 3.3.2.3); and (c) UDP glucuronosyltransferase (UDPGT: EC 2.4.1.17) which is classified as: (1) cytosol enzymes—glutathione *S*-transferase (GSH S-t: EC 2.5.1.18); and (2) membrane enzymes—γ-glutamyltransferase (GGT: EC 2.3.2.2).

GGT in plasma is the most frequently used enzyme test for proving the induction. Excluding the interfering factors, this plasma enzyme is useful as a surveillance test of hepatotoxic risk. Some endogenous metabolites modified by enzymic induction are used such as 6β-hydroxycortisol and glutaric acid; 6β-hydroxycortisol is produced from cortisol by hepatic monooxygenases. Its levels in urine are increased by many inducers (DDT, phenobarbital, and others). Glutaric acid is a product of glucuronidation. Urine levels increase after intoxication of inducers (Siest *et al.*, 1981). Maroni *et al.* (1981) studied *D*-glutaric acid (GLA) as an indicator of microsomal enzyme induction in the biological monitoring of occupational health. These authors studied the GLA concentration, corrected for creatinine, in morning urine samples of workers exposed to polychlorinated and polybrominated biphenyls. They thought that GLA urinary excretion was steady, but was affected by cigarette smoke and drugs. They recommend further investigations for increasing the precision of this test for biological monitoring. At present they see its value in occupational medicine, mainly for assessment of workers' risk on a group basis.

The methods for studying the changes in microsomal enzyme activity are indirect. The velocity of drug metabolism (antipyrine, phenylbutazone, etc.) is used as an indicator (Conney and Burns, 1972). A decrease in the half-life of phenylbutazone in workers exposed to mixtures of pesticides (mainly chlorinated) due to accelerated metabolism was found by Kolmodin-Hedman (1973). Another indirect method is the colorimetric determination of metabolites of aminopyridine in urine after oral administration (Popov and Leonenko, 1977). This method has very good results as a test for screening workers exposed to inducers (polycyclic carbohydrates, alcohols, etc.) and inhibitors (carbon disulphide, vinyl chloride, etc.) of MFOs (Popov, 1983).

4 RENAL FUNCTION

The kidney has numerous functions. Besides its basic function, excretion, it also takes part in the regulation of the electrolyte and acid–base balance, as well as producing some hormones such as erythropoietin (which stimulates erythropoiesis), renin, prostaglandins, and kinins which are related to vascular regulation.

Kidneys are target organs for many toxic substances: heavy metals and some drugs (antibiotics, analgesics, halogenated hydrocarbons, halogenated biphenyls).

With combined effects, very often one of the toxic factors does not provoke

impairment of the kidneys' function, but may alter the response of the kidneys to other substances (Hook, 1981).

Because of the concentration of toxic substances in the urine, the kidneys may suffer a toxic effect. The most frequently used tests for nephrotoxicity are blood urea nitrogen and plasma creatinine.

Biochemical indices for nephrotoxicity are described by Sunderman and Horak (1981). The authors suggest a battery of procedures that are more suitable. The measurement of urinary total proteins and assay of urine *β-N*-acetyl-*D*-glucosaminidase (NAG) activity (spectrophotometric method) are recommended since these techniques are sensitive, specific, and convenient.

Increased NAG is accepted as a very useful biochemical index in nephropathy from xenobiotics. Renal NAG (EC 3.2.1.30) activity is located mostly in the lysosomal fraction and less in the microsomal fraction. It is an indicator of tubule cells injury. β_2-Microglobulin in urine and serum radioimmunoassay are also recommended as early indicators of renal tubular damage (Sunderman and Horak, 1981).

5 EFFECTS ON REPRODUCTION

The reproductive system contains a number of sensitive targets for an occupational hazardous factor, but it is only recently that it became a topic of consideration in occupational health. Bearing in mind the evidence for the vulnerability of different stages of human reproduction to environmental influence and the recognition of reproduction as one of the most important functions of the human species, the underestimation of this problem in the past appears quite surprising. The most probable explanation for this is the difficulty in developing adequate methodology to explore the effect on the large number of complicated and interdependent events involved in the reproduction process. The final points of this process are the normal fertility of both male and female resulting in conception, successful intrauterine development, and the birth of a child without pathology including those that appear later in life as functional disorders, cancer, or reproductive failures.

In both sexes gonads have gametogenic (production of cells) and secretory (secretion of sex hormones) functions. These functions are dependent through feedback upon the hypothalamopituitary axis. The male gonads, testes, consist of two major elements: (1) seminiferous tubules in which the sperm is developed from spermatogonial stem cells (spermatogenesis), and (2) interstitium where steroids are produced by the Leydig cells. Spermatogenesis involves cell replication and mitotic and meiotic divisions yielding spermatids with a haploid number of chromosomes. The product of testicular gametogenesis is immature sperm which enters the efferent ducts and then the epididymis, where the sperm undergoes maturation processes. The vas deferens transports sperm from the distal portion of the epididymis to the urethra.

The female gonads, ovary, are the site where oogenesis takes place. In contrast to spermatogenesis, this is a totally prenatal event. The development of female germ cells is arrested at the primary oocytes (diplotene) stage. Just before ovulation, the first meiotic division is completed. One of the daughter cells, the secondary oocyte, immediately begins the second meiotic division which stops at metaphase until the sperm penetrates it. At puberty, under the influence of gonadotropins, these cells of the ovary begin to produce oestrogens and progesterone which determine the ovulatory cycle.

Conception takes place in the oviduct and starts with the penetration of sperm into the zona pellucida of the ovum and the fusion of the membrane of the ovum and sperm head. The cell division begins immediately and with the first meiotic cleavage the normal number of chromosomes is re-established. The early embryonic development proceeds through the two- and four-cell stage to the blastocyst which implants into the eroded endometrium. Then follow the stages of embryogenesis and organogenesis, and the period of maturation—foetogenesis. At birth most of the human organs are well differentiated and developed but there are still processes of growth, maturation and development to be completed, as it is with the CNS.

Reproductive outcomes with potential impairment by occupational factors can result from male exposure, but surprisingly in most countries concern has been only with the woman and pregnancy although the undoubted evidence of male gonad impairment exists. The possible toxic effects on men include: decreased libido and impotence following exposure to boron, boric acid, chloroprene, manganese, organic lead and petrol, inorganic mercury, toluene diisocyanate and vinyl chloride (reviewed by Barlow and Sullivan, 1982); impaired sperm count and morphology following exposure to dibromochloropropane (Biava *et al.*, 1978); and infertility and genetic damage resulting in spontaneous abortion and/or birth defects following exposure to antineoplastic agents, radiation, anaesthetic gases, vinyl chloride, chloroprene, chlordecone, dibromochloropropane, and dioxin (Buffler and Aase, 1982). In most of these studies the exposure has been reported to be to a single chemical but nevertheless a combination of factors could not be excluded bearing in mind that the usual situation in industry is exposure to a number of factors, and especially to the intermediates and by-products of the chemical industry. Gold *et al.* (1982) have prepared a review of studies suggesting positive associations of the father's occupation and cancer in the offspring. In particular, associations have been reported between parental occupations in hydrocarbon-, chemical-, solvent-, and motor vehicle-related occupations and brain tumours and leukemia in the offspring.

A number of clinical and epidemiological studies have demonstrated that a variety of occupational factors can alter the hormonal production of the ovaries as reflected in menstrual disturbance. Panova (1972) demonstrated this effect on the basis of epidemiological and clinical investigations, including vaginal

hormonal cytodiagnosis using the method of Dănilă-Muster *et al.* (1950), and concluded that exposure to inorganic lead in the radioelectronic industry brings about ovarian menstrual disturbances. Spasovski *et al.* (1980) reported an increased frequency of menstrual cycle disorders after occupational exposure to ethylene and ethylene oxide. With rare exceptions such as metal mercury exposure in dental nurses (Marinova., 1973), and in the production of luminescent tubes (Panova and Dimitrov, 1974), the occupational exposure has been to mixtures of chemicals such as benzene, toluene, and xylene (Michon, 1965), benzene and chlorinated hydrocarbons (Mukhametova and Vozovaya, 1972), and organic solvents and nitrocellulose polishes (Panova, 1968).

The most common effects of occupational exposure to chemicals have been reduced fertility registered as higher abortion rates and adverse effects on pregnancy such as induction of congenital malformation, higher stillbirth rates, and smaller babies. These kinds of findings were reported in the retrospective studies of Tomlin (1979) and Ericson and Kallen (1979) for women working in operating theatres. The causal relationship between anaesthetic gases does not have enough evidence to incriminate a single chemical, but more probably the combined exposure to several; the role of stress in this occupation should not be neglected. An excess risk of malformations has been reported for exposure to a variety of solvents (Holmberg, 1979) and to a mixture of unidentified chemicals in women working in laboratories during pregnancy (Blomquist *et al.*, 1981; Ericson *et al.*, 1982; Meirik *et al.*, 1979).

A significantly higher ($P < 0.05$) spontaneous abortion/birth ratio was reported for women employed in the metal industry compared with that among all Finnish women. A particular risk industry appeared to be the production of radios, television sets, and their components, probably connected with exposure to fumes from soldering resin (colophony) flux (Hemminki *et al.*, 1980).

In an epidemiological study on 576 women occupationally exposed to a mixture of different organic solvents, Panova (1974) has found an increased incidence of spontaneous abortions, stillbirths, and premature births. A higher rate of spontaneous abortions in comparison with a control group has been found among women working in laboratories without windows in the textile industry (Panova and Stamova, 1977).

Maternal exposure at conception and/or during pregnancy to the following agents has been connected with adverse effects on pregnancy outcome: ovulation stimulants, sex hormones, diethylstilboestrol, anticonvulsants, antimetabolites, tranquillizers, oral anticoagulants, diabetes, alcohol, radiation, anaesthetic gases, mercury, lead, and smoking (Buffler and Aase, (1982).

A number of industrial chemicals are shown to induce clastogenic effects in exposed populations. Sorsa (1983) and several other authors have selected among them six for which evidence of causing chromosome aberrations exists, with or without SCE induction: alkylating cytostatics, benzene, epichlorhydrin, ethylene

oxide, styrene (concentration 30 ppm), and vinyl chloride. The significance of such findings for reproductive toxicity assessment lies in the possibility that damage to human genetic material may occur not only in the somatic cells but also in the germinal cell line. Examples of a combined effect of a number of chemicals on the chromosome apparatus of human lymphocytes are the reports of Sorsa *et al.* (1982) on increased incidence of chromosome aberration in rubber industry workers, and of Pilinskaya and Lvova (1979) and Pilinskaya (1982) in pesticide workers.

The implementation of reproductive toxicity studies into the protocol for hygienic standards elaboration has become a practice in the last decade but there are still a number of MAC (maximum acceptable concentration) values established without considering this aspect. Not neglecting their value in the prediction of human risk, it is reasonable to stress that the animal studies are single exposure studies and the workers are usually subjected to combined exposures. A number of confounding factors such as infectious diseases, medical treatment and radiation, nutritional imbalance, alcohol, and tobacco might exert not only additive, but also potentiating, effects. On the other hand, very often occupational exposure is both paternal and maternal exposure, the latter being occupational, by living in the vicinity of a plant, or from polluted clothing brought home. The occupational exposure might also be combined with respect to the different stages of the reproductive process involved, for example the pre-conceptional and post-conceptional exposure of a pregnant woman.

Because of the different and often ill-defined routes and levels of exposure, risk assessment is difficult. A multidisciplinary approach with the active participation of an occupational health specialist and the planning of prospective longitudinal studies covering a number of specific parameters should be encouraged.

The newly developing field of epidemiological studies of male reproductive toxicology contains many unanswered questions, variables to be specified and techniques to be refined. However, if carefully applied, there is already available an array of techniques that can supply a reasonably firm answer (Hamill *et al.*, 1982). Such is the technique of Levine *et al.* (1980) validated on a group of workers with depressed sperm counts resulting from occupational exposure to dibromo-chloropropane (Levine *et al.*, 1981).

A prerequisite for a valid, reliable epidemiological study would be the strict determination of both group and individual type, and of the intensity and duration of occupational exposure, as well as taking into account confounding factors with proven effects on reproduction (Table 1).

Two types of studies in humans have been suggested as particularly relevant to the objectives of a male reproduction risk assessment (ORNL/EPA, 1982). The first involves surveillance studies in which periodic checking (possibly incorporated into an annual medical check-up) is done on men in a particular setting (industrial or agricultural). It might be, for example, an exposure to a reproductive toxicant in animals at high dosage but not at a dosage up to 10 times

Table 1 Potential confounding factors (factors which may independently affect the reproductive system)

Anatomical, surgical, functional, endocrinological disorders:
In men: Klinefeller's syndrome, varicocoele, cryptorchidism, vasectomy, mumps ($\geq$ 13 years), prostate infection, epididymitis
In women: tubal insufficiency, endometriosis, sperm antibodies in cervical mucus, hypothyroidism, diabetes
Infectious diseases
Drugs
Radiation (X-rays)
Alcohol
Smoking

that expected for human exposure. The following parameters are recorded yearly: questions about whether the worker has been attempting to cause a pregnancy and has been unable to do so; testicular length measurement; and measurement of serum follicle-stimulating hormone (FSH).

The second type of study would be of men exposed to a toxicant that has reproductive impact on animals at similar levels of exposure, or to high dosages of one or more general toxins for which an effect on testicular function has not been studied carefully in animals. These studies should include: (a) detailed medical, reproductive and occupational histories and physical examination; (b) analysis for volume of at least five semen samples per individual at two-day intervals, and analysis of sperm concentration, total sperm per ejaculate, sperm motility (at 37°C) expressed in terms of percentage motility, abnormalities in spermatozoal morphology, and fertility (may not be feasible for field work) such as hamster egg *in vitro* penetration test; (c) blood hormone levels: serum luteinizing hormone (LH), follide-stimulating hormone (FSH) and testosterone by radioimmunoassay; (d) gonadotropin-releasing hormone (GnRH) test (blood samples for measurements of LH and FSH are obtained before and at 30, 60, and 90 minutes after administration of GnRH (100 μg i.v.); and (e) objective data on testicular size and consistency obtained by sonography and tonometry.

Controls to be studied must be carefully chosen and matched. The following adverse effects would be expected (ORNL/EPA, 1982): (a) decreased sperm counts, motilities, and the number of sperm with normal morphology; (b) if the effects were sufficiently severe, blood testosterone levels would decrease; (c) if the toxic effects were directly on the testis, FSH levels would increase; (d) with a mild toxic effect on the testis, blood FSH levels after GnRH might exceed normal responses, even when basal FSH levels are normal; (e) if the toxic effect were primarily on the pituitary gland or CNS, LH and FSH levels would tend to decrease; and (f) the rate of infertility (number of men who have not been able to induce a pregnancy in over one year of intercourse without contraceptives) and

testicular size (with particular attention to the number of men with testicular length less than 3–5 cm) would be affected when reproductive toxicity is sufficiently severe.

In the assessment of an effect on the female reproductive function the study of menstrual cycle disorders has been repeatedly used as a sensitive indicator of an adverse occupational effect. It is recommended both as a part of yearly surveillance of women from different occupations who have no evidence of a specific impact on their reproductive system, and for specially designed epidemiological studies on women exposed to reproductive toxicants. In the second case performance of a vaginal hormonal cytodiagnostic test (Dănilă-Muster *et al.*, 1950; as used by Panova, 1976, 1978) will greatly improve the assessment of an effect on the female reproductive function; the study of the menstrual cycle (its duration, intensity, regularity) has to be included in the reproductive questionnaire for both the pre- and post-exposure periods.

Most of the attention is and should be paid to exposure during pregnancy and the subsequent outcome of pregnancy, but it should not be forgotten that maternal exposure before conception could cause spontaneous abortions and altered sex ratios (oral contraceptives) or birth defects (X-rays) and that dominant and recessive mutations may affect the germ cells of both male and female and be expressed in the offspring (foetus) or surface in future generations. On the other hand, cumulative toxicants might be a danger to the foetus even after a cessation of exposure during pregnancy.

A retrospective cohort study with registration of the pregnancy outcomes listed in Table 2 is the usual type of epidemiological study on the effect of occupation on pregnancy. It is advisable where exposures are highly heterogeneous among individuals (eventually with additional exposure to other factors) to conduct a case–control study in an occupational cohort. The power of the epidemiological study would be greatly increased.

Biological monitoring, which comprises analysis of samples taken from

Table 2 Possible reproductive outcomes resulting from parental (mother or father) occupational exposure

Absolute inability to conceive
Prolonged time to conception
Early foetal death (spontaneous abortion)
Altered sex ratios
Late foetal death
Neonatal death
Low birth weight
Birth defects
Developmental disabilities
Childhood malignancies
Childhood mortality

individuals with presumed or known exposure to a toxicant, is especially important in identifying and preventing exposure to genotoxic agents (Sorsa, 1983). In this respect the use of mutagenicity tests in analysing urine concentrations or other body fluids is non-specific with regard to the exposing agent but is specific with regard to the genotoxic character of the health hazard. The non-specificity to chemicals could be an advantage in exposures to complex (or combined) chemical mixtures such as exposure to rubber vulcanizing fumes (Falck *et al.*, 1980). The most relevant human materials for assessing human genotoxic risks are the cell samples studied with methods that allow the detection of damage induced *in vivo*. Three possible methods are currently applicable for cytogenetic monitoring (Sorsa, 1983):

(1) Cytogenetic analysis of structural chromosome aberrations in the peripheral blood lymphocytes. Prospective studies (with a sample of the individual's blood prior to exposure and its use as a control for the subsequent post-exposure samples) would be of great advantage because the interindividual variation in response to clastogen exposure could be assessed.
(2) Sister chromatid exchanges (SCEs).
(3) Micronuclei in peripheral blood lymphocytes (not routinely used).

From the available literature there are insufficient data to discuss the role of combined exposure in human reproductive toxicity. For this reason one would need: (a) data on groups of workers exposed to a certain chemical compared with workers exposed to the same, plus other, occupational factors; (b) the reports on certain occupations affecting reproduction to contain quantitative data for the intensity of the different factors; (c) specification of the exposure in relation to the different phases of the reproductive process; (d) distribution of the subjects in groups depending on the level and duration of the different agents of exposure; (e) distribution of the subjects in groups according to their medical history and their social factors which might be regarded as confounding factors, exert a synergistic reaction, or possibly potentiate the effect; and (f) intercomparison between the different parameters of reproductive toxicity (fertility, semen analysis, pregnancy outcome, cytogenicity).

Three types of comparison can be made where a cohort has been identified on the basis of their exposure to a given chemical agent of interest: (1) to the same group before and after exposure; (2) to a selected group of non-exposed workers; (3) to a reference population for whom statistics are already available; and (4) for the purpose of combined effect studies, to select groups exposed to the same agent plus other occupational factors.

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