Methods for Assessing the Effects of Mixtures of Chemicals
Edited by V. B. Vouk, G. C. Butler, A. C. Upton, D. V. Parke and S. C. Asher

Modulation of Response: Environmental Factors

E. Somers

ABSTRACT

The toxic effects of chemicals can be affected by a variety of modulating factors that can increase or reduce the chemical's effectiveness. Four particular environmental factors, namely smoking, alcohol, drugs, and climate, have been chosen for examples from the wealth of literature. Where possible the interacting effects are analysed in terms of their influence on the basic biochemical process that determines the chemical's toxicity. The examples can be used to develop protocols for the analysis of combined effects.

1 INTRODUCTION

As John Donne said so movingly, 'No man is an island, entire of itself,' and the strength of this aphorism applies equally well to consideration of the toxic effects of chemicals. Our modern technological society has produced a multitude of chemicals which can react, individually or in conjunction, to produce actual or potential threats to our health. The biochemistry of their action and hence their toxicity can be influenced by a variety of external factors—not only extrinsic physical and chemical agents but also life-style factors such as sunbathing, diet, smoking, and drinking. To delineate the modulating effects of environmental factors, I propose to discuss the influence of smoking, alcohol, drugs, and climate by drawing on particular examples that can be used to illustrate a more general theme. It must be borne in mind, however, that division into these categories is to some extent arbitrary in that there can be further interactions between these individual factors; thus alcohol can interact with drugs and drugs with alcohol, smoking can increase the risk of cardiovascular disease for women who take oral contraceptives, and so forth. These examples will show how important it is to consider the modulation of response by environmental factors in the development of methods for the assessment of the multiple effects of chemicals.

The combined action of several agents is not a new problem in pharmacology. It has been recognized for many years that there are three types of interaction:

independent, synergistic, and antagonistic. In the first type the combined action equals the sum of the effects of the individual chemicals acting independently, the final effect being additive. If the combined action of the agents, applied simultaneously or sequentially, exceeds this sum, then synergism, or potentiation, has occurred. There are variations of synergism, as, for example, the multiplicative model in which the addition of the second agent produces an effect which is proportional to the effect of the first, or when one agent which is inert itself increases the effect of the other (Saracci, 1977). When the combined action is less than expected from the sum effect, i.e. one compound reduces the potency of the other, an antagonistic effect is noted.

2 SMOKING

In 1977, Saracci analysed the then extant five epidemiological studies on the interaction between cigarette smoking and asbestos exposure in the induction of lung cancer. The multiplicative model appeared to be the most plausible, in terms of both the multistage mechanism of carcinogenesis and the results of rodent studies. However, neither asbestos exposure nor smoking was well quantified in the studies available to Saracci (1977). Further data on a large cohort of insulation workers and on Quebec chrysotile miners and millers give confirmation to a synergistic effect which is perhaps more than additive but less than multiplicative (McDonald, 1980). Nevertheless, it is clear that in absolute terms most asbestos-related lung cancers occur in those who have smoked.

A similar synergistic effect of smoking and ionizing radiation is described in the classic studies of Archer *et al.* (1973) on uranium miners. Calculation of the increase of respiratory cancer incidence due to irradiation by inhaled radon daughters gives 1.7×10^{-3} for smokers and 1.7×10^{-4} for non-smokers, per person-year at risk (UNSCEAR, 1982). The difference can be attributed to a ten-fold synergistic increase of the risk for the miners who smoke. Workers in the underground iron ore mines of northern Sweden are also exposed to radon daughters. A case–control study of the incidence of lung carcinoma in these miners by Damber and Larsson (1982) showed a synergistic effect with smoking of the multiplicative type. Synergism of this nature has, of course, important public health implications; the basic explanation of the effect is, however, less clear. A possible hypothesis is that the tobacco smoke induces non-specific effects such as changes in the production of mucus which, in turn, reduces the clearance rate of the radioactive particles resulting in a higher dose at the target cells (UNSCEAR, 1982). Attractive as this proposition is, it is impossible to be definitive at the present time.

Inorganic arsenic compounds are recognized as human carcinogens, and those occupationally exposed, such as copper smelter workers, are known to be at increased risk for lung cancer. Epidemiological studies of such workers have shown evidence of synergism between arsenic exposure and tobacco smoking

similar, in fact, to those reported for asbestos and uranium workers (Higgins *et al.*, 1981; Pershagen *et al.*, 1981). Again a multistage model for carcinogenesis would fit this type of interaction. Decreased lung clearance of arsenic, promoted by tobacco smoke, could be a contributing factor.

As a corrective to this litany on the evils of smoking it is worth observing that antagonistic effects are possible. Axelson and Sundell (1978) found a higher risk for non-smokers than for smokers in a Swedish zinc and lead mine. Weiss (1980) reported that the incidence of lung cancer in those exposed to chloromethyl ethers was greater for the non-smokers of cigarettes possibly due to the protective effect of increased mucus secretions in their bronchi. Sometimes, however, the antagonism may be more apparent than real. Neri *et al.* (1976), in a comparison of chronic respiratory disease in two Ontario cities, one pollution-free (Ottawa) and the other a mining and smelting centre (Sudbury), found the relationship between the effects of smoking and residence to be less than additive for symptomatic bronchitis but synergistic in terms of reduced respiratory function. To explain this result Neri *et al.* (1976) have postulated a ceiling effect from smoking. The public health implication is that reducing smoking, rather than ceasing, is not an effective preventive measure for chronic bronchitis as it is for lung carcinoma.

It is well known that the polycyclic aromatic hydrocarbons, particularly benzo[*a*]pyrene, present in cigarette smoke are potent inducers of microsomal enzyme activity: the average benzo[*a*]pyrene hydroxylase activity in the placentas of smokers was found to be about 60-fold higher than in the placentas of non-smokers (Conney, 1982). Cigarette smoke can thus lead to an accelerated biotransformation of drugs *in vivo* and so alter the duration and intensity of drug action. Conney and co-workers have shown that smoking markedly lowered the plasma levels of phenacetin and other workers have found that cigarette smokers have shorter plasma half-lives of antipyrine, theophylline, and caffeine (see Conney, 1982). Similarly, there are reports of more rapid elimination of heparin, propranolol, and tricyclic antidepressants by smokers as compared with non-smokers (Hansten *et al.*, 1982). Smokers also tend to have reduced serum ascorbic acid levels and hence a larger daily requirement of the vitamin (Pelletier, 1975). This reduced bioavailability in cigarette smokers could be due to antagonism from the host of chemicals present in the smoke.

3 ALCOHOL

Chronic alcoholism as a major cause of liver disease leads to abnormal drug metabolism but ethanol itself affects the biotransformation of chemicals, the nature of the effect being largely determined by the duration of exposure: acute ethanol administration depresses while chronic ingestion enhances the rate of metabolism of the foreign chemical (Hoyumpa and Schenker, 1982). The apparent paradox that the drunk are acutely sensitive to drugs while alcoholics,

when sober, are unusually tolerant of such sedatives as barbiturates and can rapidly metabolize tolbutamide, has its roots in the basic biochemistry of the interaction (Rubin and Lieber, 1971); pharmacodynamics, enzyme activity, and drug elimination are all involved.

As is well known to detective story writers, the narcotic effects of chloral hydrate are enhanced by ethanol in that, sharing a common metabolic pathway, synergism occurs thereby increasing the plasma concentrations of ethanol and trichloroethanol (Sellers *et al.*, 1972). Acute administration of ethanol can inhibit the metabolism of a foreign chemical by inhibiting the drug-metabolizing enzymes, particularly by competition for binding to microsomal cytochrome P-450 or by disturbing the lipid bilayer membrane (Hoyumpa and Schenker, 1982). Thus impaired metabolism of the benzodiazepines following short-term ethanol exposure has been ascribed by these authors to reduced hepatic clearance. Repeated exposure to ethanol, on the other hand, produces an increase of microsomal mass with increased activity of drug-metabolizing enzymes and an increase in cytochrome P-450 (Rubin and Lieber, 1971), all of which increases the elimination of drugs such as pentobarbital or meprobamate (Hoyumpa and Schenker, 1982). The opposite occurs with acute alcohol intoxication (Rubin *et al.*, 1970).

The potentiating effects of ethanol on the hepatic and renal toxicity of halogenated hydrocarbons in experimental animals has been established for many years; particular attention has been directed to carbon tetrachloride where isopropanol is an even more effective potentiator than ethanol (Traiger and Plaa, 1971). More recently Laham and Marino (1982) have found that ethanol orally administered to rats enhances the inhalation toxicity of perchloroethylene. Once again there are direct implications for the life-style of those who are exposed to these solvents in the workplace.

Although ethanol frequently potentiates toxicity of carbon tetrachloride, certain pesticides and drugs, it can also act as an antagonist and actually decrease the toxic response. Ethanol markedly reduces the toxicity of methanol and is now part of the standard treatment for methanol poisoning. Methanol is normally metabolized to formaldehyde and formate by liver enzymes and the latter metabolites are largely responsible for the toxic effects. Ethanol is preferentially metabolized and if administered it ties up the hepatic enzymes and thus prevents the appearance of formaldahyde and formate (Gleason *et al.*, 1976).

Recently, Agarwal *et al.* (1982) have found that the plasticizer di-(2-ethylhexyl)phthalate (DEHP), which can leach from finished polyvinyl chloride products, can affect the pharmacology and metabolism of alcohol. The interaction is dependent on the route of administration of DEHP (oral or intraperitoneal) and hence probably on the metabolic pathways. This is one of many examples that can be drawn of the modifying effects of alcohol on the toxicity of environmental pollutants.

A more unusual example of the potentiation effect of alcohol, this time in combination with malnutrition, was on the toxicity of cobalt to beer drinkers.

Although the beer drinkers imbibed only 1/30 of the therapeutic dose of the cobalt ion, used in this instance as a foam stabilizer, their extremely high intake of beer—approximately 10 litres a day—in conjunction with the malnutrition of their life-style resulted in a lethal cobalt toxicity. This was exacerbated by a lack of certain amino acids and led to a number of deaths from cardiomyopathy (Wiberg *et al.*, 1969). Post-mortem examination of those who died revealed a cardiac pathology similar to that found in beri-beri; however, thiamine administration to the survivors had no effects on remission of the symptoms. Subsequent biochemical studies indicated that cobalt ions complexed with α-lipoic acid. Thiamine and α-lipoic acid are essential cofactors in the pyruvate dehydrogenase and α-ketoglutarate dehydrogenase enzyme complexes which are essential components of the cellular oxidation. In essence, this deprived the heart muscle of oxygen and produced the fatalities and organ damage. It also explains the lack of response to thiamine (Grice *et al.*, 1981).

4 DRUGS

Most of us take medicines during our lifetimes and rarely are they taken in isolation. We eat food contaminated by adventitious chemicals and have residues of many environmental chemicals within us. If the effectiveness and duration of action of drugs are controlled to an appreciable extent by their metabolism by microsomal enzymes, it is apparent that any stimulation or inhibition of these enzymes will affect the drug's activity. A remarkable range of environmental chemicals, including hormones, pesticides, and carcinogens, are known to induce liver microsomal enzymes, e.g. organochlorine insecticides, urea herbicides, polycyclic aromatic hydrocarbons, and a range of food additives. Some environmental chemicals, however, inhibit microsomal functions in animals—organophosphorus insecticides and carbon monoxide, for example (Conney and Burns, 1972).

A variety of organochlorine insecticides such as chlordane, DDT, methoxychlor, dieldrin, and heptachlor have been shown to stimulate drug metabolism in rats. Similarly, workers exposed to DDT and lindane metabolized antipyrine twice as rapidly as control populations (Conney and Burns, 1972). Those occupationally exposed to large amounts of DDT had a decreased serum half-life of phenylbutazone (Poland *et al.*, 1970). The elimination rate of antipyrine in the plasma of workers occupationally exposed to polychlorinated biphenyls is significantly increased compared with controls (Krampl and Kontsekova, 1978). These effects can be ascribed to enhanced oxidative metabolism following induction of microsomal enzymes.

Pretreatment of animals with enzyme inducers such as barbiturates can protect animals from the lethal effects of such chemicals as warfarin, meprobamate, and strychnine by stimulating the metabolism of these compounds to non-toxic products (Conney, 1982). In fact, not only does the long-term administration of drugs stimulate the metabolism of foreign compounds, but the drug can stimulate

its own metabolism, for example, in dogs for phenylbutazone and tolbutamide and in man for glutethimide (Conney and Burns, 1972).

The classic example of the stimulation of one chemical's effect by another is in the two-stage model for chemical carcinogenesis where the effect of the initiating carcinogen is enhanced by the subsequent application of the promoting agent, as developed from the early studies of Berenblum (Pitot and Sirica, 1980). The fact that promotion can be modulated and even reversed, at least for skin carcinogenesis, has important implications for both disease treatment and the understanding of the biochemistry of interaction.

Tranylcypromine has been used in the treatment of psychic depression. The drug is similar in structure to amphetamine and stimulates the release of epinephrine (adrenaline). Moreover, it acts as a monoamine oxidase inhibitor and thus prevents the breakdown and elimination of the epinephrine metabolites. Excess levels of epinephrine produce a feeling of well-being and abolish psychic depression. The concomitant ingestion of certain foods such as Roquefort cheese and Burgundy wines can produce a very marked increase in blood pressure, with a strong possibility of cardiovascular damage such as heart failure or stroke. The causative agent was traced to tyramine, which is a constituent of the cheese and the Burgundy. This substance stimulates the production and release of more epinephrine and the drug prevents its destruction. The result is a marked increase in heart rate (tachycardia) and elevated blood pressure, and a possible fatal outcome (Krogh, 1983).

In fact, the literature on this subject is overwhelming and only serves to emphasize how important it is to ensure that a complete knowledge of a subject's drug regimen is known before embarking upon metabolic studies of the foreign chemicals to which he or she is exposed. To give a single example of the scope of a drug's interactions, it is salutory to examine the literature on cimetidine, the histamine H_2-receptor blocking agent which was introduced into medical practice some six years ago. It is known to inhibit the hepatic microsomal enzyme system and at the latest count is reported to interact with the following: warfarin, diazepam, benzodiazepines, chlordiazepoxide, tetracyclines, labetalol, metaprolol, propanolol, morphine, theophylline, phenytoin, carbamazepine, phenobarbitone, the 'Hemoccult' test, digitoxin–quinidine, antacids, metoclopramide, and propantheline. This list is by no means exhaustive, with further interactions with alcohol and vitamin B_{12} being noted (Anon., 1983). Of course cimetidine is a drug enjoying a runaway success but the catalogue does give some measure of the possible scope of drug interactions.

5 CLIMATE

To take the simplest parameter of climate, temperature, clearly the rate of metabolism and hence effectiveness of chemicals are temperature dependent. Examples can be given of a drug's effect being modulated by the cold—the usual

Canadian association. There is ample evidence that the detoxication of drugs and their excretion are impaired by reducing body temperature. Drugs which are conjugated with glucuronic acid in the liver undergo less conjugation and, because of reduced renal function, excretion of the drug may be reduced as the body temperature falls, particularly below 31°C. Kadar *et al.* (1982) found that reduction of body temperature markedly reduced the rate of metabolism and excretion of phenobarbital. Another interaction is the effect of a drug on the body's ability to maintain its normal thermal condition. The ingestion of alcohol followed by mild exercise in a cold environment has been shown to lead to enhanced heat loss and lower blood glucose levels (Graham, 1981).

Ionizing radiation and ultraviolet radiation are important climatic factors. There are reports of their synergistic interaction in experimental systems of microorganisms and cell lines, but not in terms of their carcinogenic action on rat skin where there may be a difference in the targets specific to the two radiations (UNSCEAR, 1982).

Natural products such as porphyrins and furocoumarins, and a wide range of drugs including chlorpromazine, tetracyclines and some oral contraceptives, can sensitize human skin to ultraviolet radiation (Magnus, 1976). The chemicals causing photosensitization may enter the body by being swallowed, injected, or absorbed through the skin. The mode of action of such a range and variety of chemicals is difficult to elucidate although the practical implications of avoiding sunburn for those taking these drugs are obvious. The Department of National Health and Welfare has, in fact, given specific advice on this question to those operating commercial tanning salons.

6 CONCLUSIONS

What are we to make then of these few examples of a myriad of possible interactions on the toxicity of a chemical? Simply, I suppose, that the Cartesian approach of reducing fields of study to their ultimate components, the discrete objects which make them up, will not serve. We have to record and integrate all the modulating influences if we are to understand the whole. The UNSCEAR (1982) report gives a useful protocol of the requirements of experimental investigations of combined effects. Emphasis is laid on (a) the exposure to the target, (b) definition of the effect particular at the level of biological complexity, whether whole-body, tissue, or cell, (c) the time sequence of the combined actions, and (d) the methodologies of analysis of the interactions. All of these must be borne in mind in the analysis of modulating effects so that we will be able to uncover ultimately the basic biochemistry of the toxic action.

ACKNOWLEDGEMENT

I am grateful to acknowledge the help of Ms Lise Mathieu in the preparation of this paper.

REFERENCES

Agarwal, D. K., Agarwal, S., and Seth, P. K. (1982). Interaction of di-(2-ethylhexyl)phthalate with the pharmacological response and metabolic aspects of ethanol in mice. *Biochem. Pharmacol.*, **31,** 3419–3423.

Anon. (1983). Interactions with cimetidine: a continuing saga. *Pharm. Int.*, January, 7–8.

Archer, V. E., Wagoner, T. K., and Lundin, F. E. (1973). Uranium mining and cigarette smoking effects on man. *J. Occup. Med.*, **15,** 204–211.

Axelson, O., and Sundell, L. (1978). Mining, lung cancer, and smoking. *Scand. J. Work Environ. Health*, **4,** 46–52.

Conney, A. H. (1982). Induction of microsomal enzymes by foreign chemicals and carcinogenesis by polycyclic aromatic hydrocarbons: G. H. A. Clowes Memorial Lecture. *Cancer Res.*, **42,** 4875–4917.

Conney, A. H., and Burns, J. J. (1972). Metabolic interactions among environmental chemicals and drugs. *Science*, **178,** 576–586.

Damber, L., and Larsson, L.-G. (1982). Combined effects of mining and smoking in the causation of lung carcinoma. *Acta Radiol. Oncol.*, **21,** 305–313.

Gleason, M. N., Gosselin, R. E., Hodge, H. C., and Smith, R. P. (1976). *Clinical Toxicology of Commercial Products*, 4th edition. Williams and Wilkins, Baltimore, Maryland.

Graham, T. E. (1981). Thermal and glycemic responses during mild exercise in +5 to −15 degrees C environment following alcohol ingestion. *Aviat. Space Environ. Med.*, **52(9),** 517–522.

Grice, H. C., Wiberg, G. S., and Heggtveit, H. A. (1981). Studies in food additive cardiopathies. In Balazs, T. (Ed.) *Cardiac Toxicology*, Vol. II, pp. 189–201. CRC Press, Boca Raton, Florida.

Hansten, P., McCormick-Pickett, N., and Natanblut, S. L. (1982). Smoking and drug interactions. *Am. Pharm.*, **NS22(9),** 32–34.

Higgins, I., Welch, K., Oh, M., Bond, G., and Hurwitz, P. (1981). Influence of arsenic exposure and smoking on lung cancer among smelter workers. *Am. J. Ind. Med.*, **2,** 33–41.

Hoyumpa, A. M., and Schenker, S. (1982). Major liver interactions: effect of liver disease, alcohol, and malnutrition. *Annu. Rev. Med.*, **33,** 113–149.

Kadar, D., Tang, B. K., and Conn, A. W. (1982). The fate of phenobarbitone in children in hypothermia and at normal body temperature. *Can. Anaesth. Soc. J.*, **29(1),** 16–23.

Krampl, V., and Kontsekova, M. (1978). Effect of polychlorinated biphenyls on the elimination rate of antipyrine from plasma of rats and man. *Bull. Environ. Contam. Toxicol.*, **20,** 191–198.

Krogh, C. M. E. (Ed.) (1983). *Compendium of Pharmaceuticals and Specialties*. The Canadian Pharmaceutical Association, Ottawa, Canada.

Laham, S., and Marino, I. (1982). Enhancement of inhalation toxicity of perchloroethylene by orally administered ethanol. *Presented at the Annual Meeting, American Industrial Hygiene Conference, Cincinnati, Ohio, June 1982.*

Magnus, I. A. (1976). *Dermatological Photobiology*. Blackwells, Oxford.

McDonald, J. C. (1980). Asbestos-related disease: an epidemiological review. In Wagner, J. C. (Ed.) *Biological Effects of Mineral Fibres*, Vol. 2, pp. 587–601. IARC Scientific Publications No. 30. International Agency for Research on Cancer, Lyon.

Neri, L. C., Pearson, R. J. C., Raman, S., and Litven, W. (1976). The risk of air pollution, work exposure, smoking and age: a study of their interaction in two Ontario communities. *Presented at the Society for Epidemiological Research, 9th Annual Meeting, Toronto, June 1976.*

Pelletier, O. (1975). Vitamin C and cigarette smokers. *Ann. N.Y. Acad. Sci.*, **258,** 156–168.

Pershagen, G., Wall, S., Taube, A., and Linnman, L. (1981). On the interaction between occupational arsenic exposure and smoking and its relationship to lung cancer. *Scand. J. Work Environ. Health*, **7,** 302–309.

Pitot, H. C., and Sirica, A. E. (1980). The stages of initiation and promotion in hepatocarcinogenesis. *Biochim. Biophys. Acta*, **605,** 191–215.

Poland, A., Smith, D., Kuntzman, R., Jacobson, M., and Conney, A. H. (1970). Effect of intensive occupational exposure to DDT on phenylbutazone and cortisol metabolism in human subjects. *Clin. Pharmacol. Ther.*, **11,** 724–732.

Rubin, E., and Lieber, C. S. (1971). Alcoholism, alcohol, and drugs. *Science*, **172,** 1097–1102.

Rubin, E., Gang, H., Misra, P. S., and Lieber, C. S. (1970). Inhibition of drug metabolism by acute ethanol intoxication. *Am. J. Med.*, **49,** 801–806.

Saracci, R. (1977). Asbestos and lung cancer: an analysis of the epidemiological evidence on the asbestos–smoking interaction. *Int. J. Cancer*, **20,** 323–331.

Sellers, E. M., Lang, M., Koch-Weser, J., LeBlanc, E., and Kalant, H. (1972). Interaction of chloral hydrate and ethanol in man. *Clin. Pharmacol. Ther.*, **13,** 37–58.

Traiger, G. J., and Plaa, G. L. (1971). Differences in the potentiation of carbon tetrachloride in rats by ethanol and isopropanol pretreatment. *Toxicol. Appl. Pharmacol.*, **20,** 105–112.

UNSCEAR (1982). *Ionizing Radiation: Sources and Biological Effects.* 1982 Report to the General Assembly, 37th Session, Supplement No. 45 (A/37/45), Annex L, pp. 727–773. United Nations, New York.

Weiss, W. (1980). The cigarette factor in lung cancer due to chloro-methyl ethers. *J. Occup. Med.*, **22,** 527–529.

Wiberg, G. S., Munro, I. C., Meranger, J. C., Morrison, A. B., Grice, H. C., and Heggtveit, H. A. (1969). Factors affecting the cardiotoxic potential of cobalt. *Clin. Toxicol.*, **2,** 257–271.