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The relationship between microsomal enzyme induction and liver tumour formation

A study on the effects of xenobiotic and naturally occurring microsomal enzyme inducers on livers of male CF-1 mice

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Abstract

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The effects of naturally occurring microsomal enzyme inducers on important hepatocellular pathways for the metabolism of foreign compounds (xenobiotics) and also upon the incidence of liver tumours in CF-1 mice treated or not with 10 mg dieldrin.kg^{-1} diet were investigated using animals maintained on semi-synthetic diet and filter paper bedding as controls. The results of the study indicate that dieldrin administration to mice results in a generalized liver enlargement predominantly due to hyperplasia. Liver enlargement in dieldrin-treated mice was followed by the appearance of nodular liver tumours, first observed at the age of 43 weeks. Conventional rodent diet and sawdust bedding were shown to contain agents that induce the microsomal mono-oxygenase system of mouse liver. However, the extent of mono-oxygenase induction by these factors was less pronounced than that caused by dieldrin. In contrast to the effects of dieldrin, conventional diet and sawdust bedding did not cause any significant induction of secondary drug-metabolizing enzyme systems, e.g. epoxide hydratase, glutathione *S*-epoxide transferase and UDP-glucuronyl transferase. Histopathological examination of livers demonstrated a low incidence of tumours in the livers of mice not treated with dieldrin. These tumours were generally benign in character although a few showed morphological characteristics associated with malignant liver cell tumours. The overall incidence of liver tumours was significantly increased in dieldrin-treated animals. Both benign and malignant liver tumours were found in dieldrin-treated mice; the latter type of lesion showing evidence of lung metastasis. Conventional diet and sawdust bedding did not exert any obvious influence on the development of 'spontaneous' tumours in the livers of male CF-1 mice.

It is concluded that microsomal enzyme inducers such as dieldrin act by facilitating the expression of a pre-existing oncogenic factor, probably by inducing hyperplasia.

Free descriptors: liver enlargement, environmental components, dieldrin, drug-metabolizing enzymes.

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Abbreviations

AAF	2-acetylaminofluorene
BHC	benzenehexachloride (all isomers) = HCH
BHT	butylated hydroxytoluene
CD	conventional rodent diet
CPA	cyproterone acetate
D	10 mg dieldrin.kg^{-1} diet
DDT	dichlorodiphenyltrichloroethane
DNA	deoxyribonucleic acid
DTNB	5,5'-dithiobis-(2-nitrobenzoic) acid
F	filter paper bedding
G-6-Pase	glucose-6-phosphatase
G-6-PDH	glucose-6-phosphate dehydrogenase
GSH	glutathione
γ-GT	γ-glutamyltranspeptidase
HCB	hexachlorobenzene
HCH	hexachlorocyclohexane (all isomers) = BHC
HEOD	dieldrin
3-MC	3-methylcholanthrene
NADPH	nicotinamide-adenine dinucleotide phosphate
NS	not significant
PCA	perchloric acid
PCB	polychlorinated biphenyl(s)
PCN	pregnelonone-16α-carbonitrile
RLW	relative liver weight, i.e. liver weight.100 g^{-1} bodyweight
RNA	ribosenucleic acid
S	sawdust bedding
SER	smooth, i.e. ribosome-free endoplasmic reticulum
SSD	semi-synthetic diet
TCA	trichloroacetic acid
TCDD	2,3,7,8,-tetrachlorodibenzo-p-dioxin
TX-100	octylphenoxypolyethoxyethanol
UDPGA	uridine-diphosphoglucuronic acid

1 Introduction

1.1 OBJECTIVES OF THE STUDY

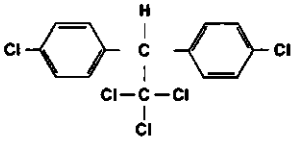
A variety of xenobiotic compounds are known to induce characteristic changes in the livers of laboratory animals. These changes include liver enlargement (hepatomegaly), usually as a result of cell enlargement (hypertrophy) or cell replication (hyperplasia), induction of drug metabolising enzymes, and proliferation of the smooth endoplasmic reticulum (SER). Such changes may not be accompanied by evidence of liver damage and in such cases are reversible upon withdrawal and elimination of the compound (Schulte-Hermann et al., 1971; Schulte-Hermann, 1974a; Wright et al., 1972, 1977; Depierre & Ernster, 1976; Bolender & Weibel, 1973; Böhm & Moser, 1976).Consequently, most authors regard this phenomenon as an adaptive response of the organ to increased functional demands. However, chronic exposure of various strains of mice to dieldrin (HEOD), phenobarbitone, DDT and α-, β- and γ- stereoisomers of hexachlorocyclohexane (HCH, also known as benzenehexachloride, BHC) may lead to the development of liver tumours (Davis & Fitzhugh, 1962; Walker et al., 1973; Thorpe & Walker, 1973; Tomatis et al., 1972, 1974; Turosov et al., 1973; Terracini et al., 1973a, 1973b; Peraino et al., 1973a; Ponomarkov and Tomatis, 1976; Nagasaki et al., 1971, 1972; Ito et al., 1973).

There is no apparent relationship in chemical structure between these compounds (Figure 1). Their main common features are that they are lipophilic at a physiological pH and induce the microsomal mono-oxygenase system of mammalian liver (Conney, 1967). This latter feature has led to the suggestion that a common property of microsomal enzyme inducers may be to enhance the incidence of liver tumours in susceptible animal species (Wright et al., 1972, 1977).

Present experimental evidence supports the hypothesis that these compounds act by facilitating or exacerbating the expression of pre-existing oncogenic factors in susceptible animal species. Reports that microsomal enzyme inducers, such as phenobarbital, DDT, α-HCH and butylated hydroxytoluene (BHT) may also promote the formation of rat liver tumours from lesions previously initiated by liver carcinogens (Peraino et al., 1971, 1973b, 1975, 1977; Schulte-Hermann, 1978) are consistent with this hypothesis.

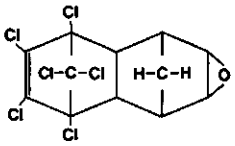
The contention that xenobiotic microsomal enzyme inducers may promote rather than initiate liver tumourigenesis is also supported by the observation that strains of mouse with a spontaneous incidence of liver tumours, e.g. CF-1 and C3H mice, are particularly susceptible to the tumourigenic effects of microsomal enzyme inducers (Walker et al., 1973; Thorpe & Walker, 1973; Thorpe & Hunt, 1975; Tomatis et al., 1972, 1974; Turosov et al., 1973; Peraino et al., 1973a).

Several reports (Ferguson, 1966; Vessel, 1967; Loub et al., 1975; Babish & Stoesand, 1975, 1977) indicate that diets and bedding employed in toxicological studies with rodents



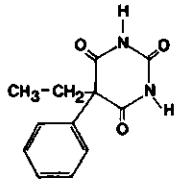
dichlorodiphenyl trichloroethane

DDT



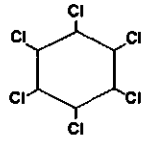
endo-exo isomer of 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4,5,8-dimethano naphtalene

Dieldrin
HEOD



5-ethyl-5-phenyl-2,4,6-(1H,3H,5H,)pyrimidine - trione

Phenobarbitone
Phenobarbital
Luminal



hexachlorocyclo-hexane

benzenehexa-chloride

HCH
(all isomers)

BHC
(all isomers)

Lindane
(γ-isomer)

Fig. 1. Chemical formula, nomenclature and trade name(s) of microsomal enzyme inducers shown to be tumourigenic in mouse liver.

may contain naturally occurring and, possible, adventitious microsomal enzyme inducers. If apparently unrelated xenobiotic microsomal enzyme inducers can enhance the incidence of liver tumours in various strains of mice, similar effects might be expected as a consequence of exposure to such naturally occurring or adventitious microsomal enzyme inducers in the animals' environment. Accordingly, the principal objectives of the current study were to determine the capacity of diets and bedding employed in this laboratory, to induce microsomal mono-oxygenases and related enzyme systems and to study the relationships between these effects and tumour incidence in the livers and other tissues of a susceptible strain of mouse, CF-1.

The effects of a conventional rodent diet (CD) and of bedding material, e.g. softwood sawdust (S), were studied using animals maintained on semi-synthetic diet (SSD) and filter paper bedding (F) as controls. The administration of 10 mg dieldrin.kg^{-1} diet to some of the experimental treatment groups served as a positive control, i.e. a potent microsomal enzyme inducer with tumourigenic properties in various strains of mouse.

In addition, commercial diet may contain traces of carcinogenic agents, e.g. nitrosamines and aflatoxin B_1, (Schoental, 1974) and softwood sawdust is frequently contaminated with certain wood preservatives, e.g. pentachlorophenol, dieldrin, HCB, DDT or endrin (Baldwin, unpublished observations). The presence of highly toxic and carcinogenic substan-

ces in the animals' environment might be a major cause of the development of 'spontaneous' liver tumours in tumour-susceptible strains of mouse. As a result, the administration of a purified semi-synthetic diet and maintenance on purified bedding, such as shredded filter paper, might significantly reduce the initiation of liver carcinogenesis in tumour-susceptible strains of mouse. It was also decided to study the possible implications of such effects in mice treated with a potent microsomal enzyme inducer (dieldrin). The studies with this latter compound served as both a positive control and as an aid to perspective. The experiments entailed both biochemical investigations of the liver and histopathological assessment of the incidence of liver tumours in the various treatment groups.

1.2 INDUCTION OF LIVER ENLARGEMENT BY XENOBIOTIC AGENTS

Many drugs, insecticides, food additives and other chemicals are known to induce liver enlargement (Barka & Popper, 1967; Schulte-Hermann, 1974a). The chemical structures of the substances that induce liver enlargement vary widely and their only common feature is their lipid solubility at a physiological pH. Furthermore, many inducers of liver enlargement are substrates of the microsomal mono-oxygenase system of mammalian liver and are able to induce the activity of these enzymes (Conney, 1967; Schulte-Hermann, 1974a; Wright et al., 1977).

The quantities of xenobiotic inducers required to produce a measurable enlargement of the liver vary widely. Threshold doses observed with some compounds are shown in Table 1. It should be noted that a considerable variation of liver sensitivity has been observed from one study to the other (Hodge et al., 1967). In most of the studies reported so far, the increment of liver weight ranged from 10% to approximately 50%, but increases of 100% or more have been reported (Fitzhugh & Nelson, 1947; Fitzhugh et al., 1950; Kunz et al., 1966a; Schlicht et al., 1968; Schulte-Hermann et al., 1974b). The capacity of a compound to induce liver enlargement is related to the rate of its elimination from the body. Kunz and co-workers (1966a) showed that hexobarbital, which is rapidly metabolized and excreted, produced only a small gain in liver weight, whereas the long-acting barbiturates phenobarbital and N-methylphenobarbital led to striking increases in relative liver weight (i.e. liver weight per 100 g bodyweight, RLW).

In the course of liver enlargement, the proportions of the main cell constituents water, protein, lipid, glycogen and RNA appear to remain unchanged. This has been shown in experiments in rats and mice treated with phenobarbital (Conney et al., 1960; Kunz et al., 1966b; Schlicht et al., 1968; Agryris & Magnus, 1968), halothane (Kunz et al., 1966b), thiourea (Doljanski et al., 1956), α-HCH (Schlicht et al., 1968), pyrethrum (Springfield et al., 1973), BHT (Pascal et al., 1970), or polycyclic aromatic hydrocarbons (Arcos et al., 1961; Argyris & Layman, 1969).

Enzymic profiles during the course of xenobiotic-induced hepatomegaly have also been studied. Stier et al. (1972) and Kunz & Schnieders (1970) measured the activities of several mitochondrial, microsomal and hyaloplasmic enzymes during the course of liver enlargement in rats exposed to barbiturates and halothane. Most enzymes catalysing intermediary metabolism increased in proportion to liver size. However, microsomal NADPH-oxidase

was more than proportionally increased after phenobarbital treatment and this finding was considered indicative of stimulation of drug-metabolising enzymes.

The cellular composition of the enlarged liver was analysed by quantitative histological procedures. Kunz et al. (1966b) and Preis et al. (1966) observed that parenchymal space increased from 82 to 87% and that the extra-parenchymal space was reduced from 18 to 13% in the livers of phenobarbital-treated mice. Likewise, rat liver also showed decreases in extrahepatocytic space after phenobarbital treatment (Stäubli et al.,1969). These studies indicate that the parenchymal fraction contributed to a predominant extent to the enlargement of the liver by the compounds tested.

Several inducers of liver enlargement have been shown to induce disproportionate increases of certain hepatocyte organelles. Remmer & Merker (1963) demonstrated that the exposure of rats to phenobarbital leads to a considerable augmentation of the smooth, i.e. ribosome-free, membranes of the endoplasmic reticulum (SER). Subsequent studies revealed that hepatocellular SER was increased by many of the compounds that induce liver growth, including α-HCH (Koransky et al., 1966), DDT (Ortega, 1966), chlordane (Fouts & Rogers, 1965), BHT (Lane and Lieber, 1967; Botham et al., 1970), dieldrin (Wright et al., 1972), and others (Meldolesi, 1967). In some studies (Ortega, 1966; Meldolesi, 1967; Wright et al., 1972) formation of concentric whorls of SER was observed. A quantitative electron microscopic analysis of the liver of phenobarbital-treated rats, performed by Stäubli et al. (1969), revealed the quantitative importance of cytoplasmic changes in liver enlargement with endoplasmic reticulum accounting for more than half of the increase in cytoplasmic volume.

1.2.1 Hyperplasia and hypertrophy

Liver enlargement may involve an increase in cell size or cell number or a combination of the two. The terms *'hypertrophy'* and *'hyperplasia'* are commonly used to describe these events. Conventionally, 'hypertrophy' means an increase in cell volume and 'hyperplasia' an increase in cell number. However, this morphological definition has limited usefulness in the liver, which contains cells of different ploidy (Barka & Popper, 1967; Epstein et al., 1967). Enlargement of a cell, without changes in cell ploidy, clearly represents hypertrophy. However, when cell enlargement is associated with an increase in cell ploidy - which requires DNA replication - there is no change in the ratio of nuclear volume to cytoplasmic volume and the only deviation from conventional hyperplasia is the absence of cell division, i.e. an increase in ploidy may be regarded as an arrested form of cell replication.

For this reason, Barka & Popper (1967) have defined hypertrophy as an increase in cell size *without* an increase of cell ploidy and hyperplasia as any increase in genetic material of the liver, whether derived from an increase in cell ploidy or from cell division. This definition simplifies the description of liver enlargement. In morphologic terms, the characteristic of hypertrophy is a decrease in the ratio of nuclear volume to cellular volume; in biochemical terms it is a relatively decreased DNA concentration per cell. Hyperplasia per se, on the other hand, is characterised by a constant ratio of nuclear and cellular volume and a constant DNA concentration.

The relative contribution of hypertrophy and hyperplasia to chemically induced liver enlargement appears to depend on various factors, such as the dose and properties of the inducer, and species and strain of the animals. In studies with rats, dieldrin and phenobarbital are reported to induce predominantly liver cell hypertrophy, while α-HCH and BHT elicited predominantly hyperplasia (Wright et al., 1972; Schulte-Hermann, 1971, 1974a, 1974b, 1979). The results of studies with dieldrin and phenobarbitone in various species (Wright et al., 1972, 1977, 1978) indicated that liver cell hypertrophy occurred in rats, mice and dogs exposed to these compounds. In the case of dieldrin-treated rhesus monkeys, only the first indications of hepatocellular hypertrophy, as evinced by marginal increases in microsomal protein were present in the absence of obvious liver enlargement. No increases in the DNA content of the liver were detected in rats or dogs when these animals were exposed to high doses of dieldrin or phenobarbital, indicating that hyperplasia does not make a significant contribution to the dieldrin- or phenobarbitone-induced liver enlargement in these species. Total liver DNA was also unaltered in rhesus monkeys fed dieldrin at concentrations of up to 5 $mg.kg^{-1}$ for 6.5 years (Wright et al., 1978). In the mouse, however, liver DNA content was increased from the outset of exposure to both dieldrin and phenobarbital. Hyperplasia and hypertrophy were reported to make a similar contribution to the overall enlargement of the liver.

1.2.2 Intralobular differences

Fitzhugh and his associates (1947, 1950) reported that administration to rats of DDT or α-HCH led to marked enlargement of centrilobular hepatocytes while periportal cells were not enlarged. Similar observations were made later in studies with DDT (Ortega, 1966; Thorpe & Walker, 1973), other chlorinated hydrocarbon insecticides (Ortega et al.,1957) and phenobarbital (Kunz et al., 1966b). Increases in diameter or number of nuclei were not observed in the centrilobular cells (Fitzhugh & Nelson, 1947; Fitzhugh et al., 1950; Kunz et al., 1966b) indicating that hypertrophy was the cause of enlargement rather than increased ploidy.

Electron microscopic analysis revealed that proliferation of SER occurred predominantly in the vicinity of the central vein of the liver lobule (Burger & Herdson, 1966; Becker & Lane, 1968). These observations support findings (Stäubli et al., 1969) indicating that SER multiplication is an important factor in cell hypertrophy after phenobarbital administration.

While hypertrophy predominates in the centrilobular area, proliferating cells have been found in all parts of the lobule, but preferentially in the periportal and midzonal areas (Grisham, 1973).

1.2.3 Dose-dependence

The extent of liver enlargement clearly depends on the dose of the inducer used. This relationship has been demonstrated in studies with barbiturates (Kunz et al., 1966a), pyrethrum (Springfield et al., 1973), α-HCH (Schulte-Hermann et al., 1974b), DDT (Hoffman et al., 1970), BHT (Gilbert & Golberg, 1965) and dieldrin (Wright et al., 1972).

Above the range of the respective threshold doses, a linear relationship appears to exist between the increase of liver mass and the logarithm of the dose. This relationship appears valid when the inducing compound is administered only once (Schulte-Hermann et al., 1974b) but also upon daily administration for several days or weeks (Kunz et al., 1966a; Hoffman et al., 1970; Gilbert & Golberg, 1965). Likewise, hepatic DNA is increased in proportion to the logarithm of the dose (Schulte-Hermann, 1974b). Toxic effects of the inducers have limited attempts to determine maxima of the growth responses of the liver (Kunz et al., 1966a; Hoffman et al., 1970; Schulte-Hermann et al., 1974b). Studies with α-HCH (Schlicht et al., 1968; Schulte-Hermann et al., 1974b) revealed that this compound can increase RLW by more than 100%. An even higher increase in RLW, i.e. almost 200%, was observed in rats bearing pituitary tumours that excreted excessive amounts of pituitary hormones (Epstein et al., 1967; Milkovic et al., 1964).

1.2.4 Reversibility of liver enlargement

Liver weight returns to normal when the administration of the inducing substance ceases. This observation was made in studies with barbiturates (Kunz et al., 1966a; Schlicht et al., 1968; Owen et al., 1971), pyrethrum (Springfield et al., 1973), α-HCH (Schlicht et al., 1968; Schulte-Hermann et al., 1971), DDT (Fitzhugh & Nelson, 1947), dieldrin (Ferrigan et al., 1965) and BHT (Schulte-Hermann et al., 1971; Gilbert & Golberg, 1967).

The rate at which liver enlargement recedes seems to be closely related to the rate of elimination of the inducer. Hence, liver weights in rat and mouse return to normal levels within a few days when compounds with relatively short biological half-lives, such as phenobarbital (Kunz et al., 1966a; Schlicht et al., 1968) or BHT (Gilbert & Golberg, 1967) are used. A return to normal liver weight may take weeks when chlorinated hydrocarbons such as DDT (Fitzhugh & Nelson, 1947) or α-HCH (Schlicht et al., 1968) are used.

The increased amount of endoplasmic reticulum also returns to normal when administration of the inducer is discontinued (Bolender & Weibel, 1973). Excess membranes, induced by phenobarbital treatment, were removed within 5 days after the end of treatment. During the regression phase an increase in the number of autophagic vacuoles occurred which suggests that, in addition to a biochemical turnover, specific cellular mechnisms may be responsible for the bulk-removal of phenobarbital-induced membranes (Bolender & Weibel, 1973).

Conflicting results have been reported on the fate of the excess of liver cells (due to hyperplasia) in the regression period. After discontinuation of phenobarbital treatment of rats, the increased number of nuclei was found to be reduced (Argyris & Magnus, 1968). In contrast, experiments with α-HCH and BHT indicated that the elevation of the total DNA content persisted throughout the period of regression of the increased liver size (Schulte-Hermann et al., 1971). Schulte-Hermann (1974a) suggested that the apparent reduction in the differences between the liver DNA content of control and experimental animals, observed in some studies, might be due to developmental growth of control livers during the regression period. It would seem that, in contrast to other changes in the li-

ver, there is no certainty at present about the fate of the induced excess of liver cells during regression.

1.3 ENZYME INDUCTION BY XENOBIOTIC COMPOUNDS

1.3.1 Mono-oxygenase

The oxidative catabolism of lipophilic substrates is catalysed by an enzyme system which requires both NADPH and molecular oxygen and is designated mono-oxygenase(s) or mixed-function oxidase(s). This membrane-bound system is one of the most versatile enzyme complexes known. It metabolizes not only endogenous substrates such as steroids and fatty acids but also a variety of foreign compounds, e.g. drugs, insecticides, and carcinogens. Reactions catalysed include aromatic and aliphatic hydroxylation, *N*-, *O*-, and *S*- dealkylation, sulfoxidation, deamination, epoxidation, desulfuration and dehalogenation (Conney, 1967; Gillette et al., 1972).

The system consists of a flavoprotein referred to as NADPH → cytochrome c-reductase and a hemoprotein (cytochrome); in addition phosphatidyl-choline is required for catalytic activity (Lu et al., 1969; Lu & Levin, 1974). Substrate specificity resides in the hemoprotein moiety, and it has been found that at least six different forms of these cytochromes exist in the liver which differ in catalytic activity towards various substrates as well as in molecular weight and in immunological and spectral properties (Thomas et al., 1976). On the basis of spectral properties the hemoproteins are frequently referred to as cytochrome P-450 or cytochrome P-448. It is quite likely that the activity of each of the various hemoproteins is under different genetic control and may, therefore, be influenced differently by various groups of xenobiotic inducers.

The concentration of cytochrome P-450 exceeds that of the mitochondrial cytochromes (Estabrook et al., 1971) and comprises approximately 1% of the total liver proteins (Schulte-Hermann, 1974a). Cytochrome P-450 may increase several-fold after treatment with xenobiotic compounds (Conney, 1967).

There are two 'classical' groups of xenobiotic inducers of hepatic mono-oxygenases. The first group, represented by phenobarbital, stimulates the degradation of many substrates (e.g. *N*-demethylation of aminopyrine, ethylmorphine, benzphetamine), the second, exemplified by 3-methylcholanthrene (3-MC), stimulates the hydroxylation of benzpyrene but has little or no effect on most other oxidative pathways (Conney, 1967; Gillette et al., 1972). These observations have now been explained by the preferential synthesis of catalytically different cytochromes (P-450 and P-448) induced by the two groups of inducers (Haugen & Coon, 1976a; Haugen et al., 1976b; Thomas et al., 1976).

More recent evidence suggests that there are probably more than two groups of microsomal enzyme inducers. Thus, ethanol was found to stimulate microsomal mono-oxygenase(s) with a high capacity for aniline hydroxylation (Villeneuve et al., 1976). Pregnelenone-16α-carbonitrile (PCN) and other steroids may represent another group of enzyme inducers (Lu et al., 1972) and polychlorinated biphenyls (PCB) and hexachlorobenzene (HCB) produce a pattern of induction that resembles a mixture of the changes produced by phenobarbital and 3-MC (Alvares et al., 1973; Stonard & Greig, 1976).

1.3.2 Epoxide hydratase, glutathione transferase and UDP-glucuronyl transferase

Induction of hepatic mono-oxygenases is frequently associated with increased activities of other enzyme systems, which also serve to metabolise lipophilic substrates.

Epoxide hydratase catalyses the hydration of epoxides, highly reactive and toxic intermediates which may arise from aromatic hydrocarbons and other substrates by mono-oxygenase action. Hepatic epoxide hydratase activity is elevated substantially by pre-treatment of rats and mice with phenobarbitone and to a lesser extent by pre-treatment with 3-MC (Oesch et al., 1971, 1973). PCN has also been reported to induce a slight increase in hepatic epoxide hydratase activity (Oesch, 1975).

The glutathione transferases which employ glutathione (GSH) as a co-substrate also play a prominant role in the inactivation of toxic intermediates. A broad spectrum of structural types may undergo spontaneous or enzyme-mediated conjugation with glutathione in vivo leading ultimately to the formation of *N*-acetyl cysteine conjugates (mercapturic acids) which may be excreted via the bile or as urinary metabolites (Boyland & Chasseaud, 1969; Chasseaud, 1973, 1976). Rat liver cytosol contains at least 6 different GSH transferases of broad and overlapping specificities, including the anion-binding ligandin or transferase B (Jakoby et al., 1976).

Glutathione transferases are inducible by common inducers of hepatic mono-oxygenases, such as phenobarbital, TCDD (2,3,7,8-tetra chlorodibenzo-p-dioxin) and PCN (Klassen & Plaa, 1968; Darby and Grundy, 1975; Kaplowitz et al., 1975; Jenna & Bend, 1977).

The liver microsomal UDP-glucuronyl transferase system constitutes another group of enzymes which can be induced by treatment with foreign compounds. Glucuronidation is a major pathway by which the body inactivates and eliminates a wide variety of lipid-soluble endogenous and exogenous compounds such as phenols, carboxylic acids, aliphatic and aromatic alcohols and certain aromatic amines (Dutton, 1966). There is accumulating evidence for the existence of a number of UDP-glucuronyl transferases each possessing different substrate specificities. These enzymes can be selectively induced by different types of inducing agents. For example, treatment of rats with phenobarbital induces the glucuronidation of chloramphenicol and bilirubin whereas pretreatment of the animals with 3-MC induces the glucuronidation of 1-naphthol and *p*-nitrophenol (Dutton, 1966; Bock et al., 1973). Interpretation of these data is somewhat complicated by the latency of the enzymes, i.e. glucuronyl transferases can be activated up to 10-fold in vitro by addition of detergents (Bock & White , 1974). Latency may be due to conformational restraints within the intact endoplasmic reticulum.

1.4 INDUCTION OF LIVER TUMOURS BY MICROSOMAL ENZYME INDUCERS

1.4.1 Carcinogenicity studies in mice

The first carcinogenicity studies conducted in mice exposed to a microsomal enzyme inducer (dieldrin) were reported by Davis & Fitzhugh (1962). The results of this study were inconclusive because the majority of animals were not available for pathological examination. However, in a second study, the feeding of 10 mg dieldrin.kg^{-1} diet shortened

the life-span of C3HeB/Fe mice by 2 months and increased the incidence of liver tumours. It should be noted, however, that the latter study showed a very poor survival time (average survival time: 51.4 weeks in treated mice compared with 59.8 weeks in controls) and that a substantial proportion of the animals was discarded at autopsy: 70/218 dieldrin-treated mice and 83/217 control animals. Thus, these experiments failed to provide conclusive evidence on the tumourigenicity of the compound in this species.

Another series of experiments with dieldrin in the CF-1 mouse were reported by Walker et al. (1973) and Thorpe & Walker (1973). In the main experiment, 87 - 297 mice of each sex were fed diets containing either 0, 0.1, 1.0 or 10.0 mg dieldrin.kg^{-1} for 132 weeks (Table 2). Fifty percent mortality was reached at 15 months among mice fed 10 mg dieldrin.kg^{-1} diet and at 20 months in the other groups. The liver tumour incidence (Table 2) was enhanced in all of the three dieldrin treatment groups. The highest incidence of liver tumours was observed in mice exposed to 10 mg dieldrin.kg^{-1} diet: 94% in males and 92% in females. CF-1 mice fed on 0.1 and 1.0 mg dieldrin.kg^{-1} diet showed a similar incidence of liver tumours (25-35% in both males and females). The results of a second dose-response study, in which exposure lasted for 128 weeks, showed that the incidence of liver tumours was enhanced in all of the five dieldrin treatment groups (1.25, 2.5, 5.0, 10.0 and 20.0 mg dieldrin.kg^{-1} diet, both in male and female CF-1 mice).

Neither of these studies with dieldrin in the CF-1 mouse produced a clear-cut dose-response relationship. However, it was clearly demonstrated that high doses of dieldrin invariably induced a high incidence of liver tumours. Furthermore, clear dose-response relationships may never be established when the survival of the animals is adversely affected by continuous exposure of mice to high doses of the test compound. On the other hand, it is interesting to note that, in the main experiment (Table 2), dieldrin enhanced the incidence of liver tumours when ingested at a dietary concentration of 0.1 mg kg^{-1}. This concentration is below the reported threshold dose for the induction of liver enlargement (Fitzhugh et al., 1964; Walker et al., 1969).

It would seem, therefore, that even though high doses of dieldrin are required to produce a maximum increase of the incidence of liver tumours in CF-1 mice, this compound may exert tumourigenic effects in livers of this strain of mouse at very low levels of exposure.

In a subsequent study (Walker et al., 1973), groups of male CF-1 mice were fed on 10 mg dieldrin.kg^{-1} diet for up to 64 weeks and allowed to live until 104 weeks. The results of this study (Table 3) show that short-term exposure of mice to dieldrin, e.g. 8 weeks, produced a highly significant increase in the incidence of liver tumours (40% in treated mice versus 11% in controls). However, the highest incidence of liver tumours (100%) was found in mice receiving the compound for the longest period of time (64 weeks).

In an unpublished experiment (Thorpe & Hunt, 1975) a study was made of the pathological changes in three strains of mice (CF-1, LACG and CF-1 x LACG) following chronic dieldrin administration (10 mg.kg^{-1} in the diet). The results of this study (Table 4) suggest considerable strain differences in susceptibility to the tumourigenic effects of dieldrin. Hybrid (LACG x CF-1) mice responded with liver tumour formation in a somewhat similar fashion to CF-1 mice, but fewer liver tumours were found in treated LACG mice and in males the increased risk was only marginally significant.

Among several hundred mice with liver tumours, metastases were found in only 15. Unpublished studies (Thorpe, 1973) showed that liver cell tumours from mice that had received prolonged oral exposure to dieldrin were capable of autonomous growth as subcutaneous transplants without recourse to the use of immuno-suppressive agents. This latter finding was highly suggestive of the malignant character of the liver tumours.

In recent years, a number of reports have been published showing that certain other microsomal enzyme inducers also exerted tumourigenic effects on mouse liver.

A 2-generation dose-response study on the feeding of DDT to CF-1 mice involving a total of 881 treated and 224 control mice was reported by Tomatis et al. (1972). Dietary concentrations of 2, 10, 50 and 250 mg technical DDT.kg^{-1} were administered for life-span. In both parent (P) and F_1 generation mice an increased incidence of liver tumours was observed in all males exposed to DDT particularly in those exposed to the highest concentration (Table 5). The administration of 2 and 10 mg DDT.kg^{-1} diet to parent and F_1 generation female mice, however, did not result in a significant excess over control levels of liver-cell tumours. An incidence of 13% was observed in (P + F_1) females given 50 mg DDT.kg^{-1} diet (significant at the 5% level only). A high incidence of liver tumours was observed in females of both generations exposed to 250 mg DDT.kg^{-1} diet (63% in P and 71% in F_1). A later study by the same group of workers, reporting on the effects of DDT on 6 consecutive generations of CF-1 mice (Turosov et al., 1973), confirmed these results.

The results of both studies with DDT in the CF-1 mouse (Tomatis et al., 1972; Turosov et al., 1973) suggest that the tumourigenic potential of this compound is more easily expressed in males than in females. However, it is interesting to note that in these experiments females showed a lower background incidence of liver tumours. In experiments with CF-1 mice showing no clear sex difference in spontaneous liver tumour incidence (Walker et al., 1973; Thorpe & Walker, 1973), the tumourigenic effects of DDT were found to be similar in males and females.

A 2-generation study with DDT involving a total of 515 female and 431 male BALB/c mice was reported by Terracini et al. (1973a, 1973b). DDT was administered at dietary concentrations of 2, 20 or 250 mg.kg^{-1} for life-span. A comparison of the results from this study with those from studies conducted with DDT in the CF-1 strain of mouse (Tomatis et al., 1972; Turosov et al., 1973; Walker et al., 1973; Thorpe & Walker, 1973) strongly suggests that the latter strain, which generally exhibits a relatively high incidence of spontaneous liver tumours, is more susceptible to the tumourigenice effects of DDT than BALB/c mice. The occurrence of spontaneous liver tumours in BALB/c mice is rare (Andervont & Dunn, 1948; Deringer, 1965; Madison et al., 1968; Smith & Pilgrim, 1971; Terracini et al., 1973a).

In more recent experiment, reported by Tomatis et al. (1974), groups of 60 male and 60 female CF-1 mice were given 250 mg DDT.kg^{-1} diet for 15 or 30 weeks after which the mice were killed at different time intervals (at 65, 95 and 120 weeks after initiation of the experiment. The results of this study are similar to those reported by Walker et al. (1973) on the tumourigenicity of dieldrin in the CF-1 mouse and suggest that a limited period of exposure to microsomal enzyme inducers results in an increased appearance of liver tumours similar to that caused by life-span exposure. The shorter the period of ex-

posure the lower the incidence of liver tumours. In this context, it is interesting to note that life-span exposure of CF-1 mice to 250 mg DDT.kg^{-1} diet resulted in a higher incicence of liver tumours than 30-weeks exposure.

Several recent carcinogenicity studies with phenobarbitone have shown that this 'classical' microsomal enzyme inducer may also enhance the incidence of liver tumours in mice (Table 6). This effect has been demonstrated in strains of mice that are known to be susceptible to 'spontaneous' development of liver tumours, e.g. CF-1 mice (Thorpe & Walker, 1973; Ponomarkov et al., 1976) and C3H mice (Peraino et al., 1973a).

Similarly, several stereo-isomers of HCH have now been found to possess tumourigenic potential in mice (Table 7). The results of two studies by Ito and co-workers indicate that α-HCH may induce liver tumours in male dd mice within 6 months of exposure (Nagasaki et al, 1971, 1972; Ito et al., 1973). This group found no evidence for tumourigenic potential of other stereo-isomers. However, a study by Thorpe & Walker (1973) showed that β- and γ-HCH may enhance the incidence of liver tumours in CF-1 mice.

CONCLUSIONS

The carcinogenicity studies discussed above have clearly established that various microsomal enzyme inducers, such as dieldrin, DDT, phenobarbitone, α, β and γ HCH are tumourigenic in livers of various strains of mouse.

The tumourigenic effects of microsomal enzyme inducers have been shown to be pronounced in strains of mouse that show a relatively high incidence of spontaneous liver tumours (Thorpe & Walker, 1973; Walker et al., 1973; Tomatis et al., 1972; Turosov et al., 1973; Peraino et al., 1973a; Ponomarkov et al., 1976) whereas strains of mouse with a low background incidence of liver tumours appear to be less susceptible (Thorpe & Hunt, 1975; Terracini et al., 1973a, 1973b).

The tumourigenic effects of the inducer were most pronounced when high doses of the compound were used. Moreover, a positive relationship appears to exist between the duration of treatment with an inducer and the incidence of liver tumours (Walker et al., 1973; Tomatis et al., 1974). On the other hand, it has been demonstrated that microsomal enzyme inducers may enhance the incidence of liver tumours at exposure levels below the threshold doses for the induction of liver enlargement (Walker et al., 1973; Turosov et al., 1973; Tomatis et al., 1972).

Therefore it would seem that some compounds exert tumourigenic effects at very low levels of exposure, even though maximum enhancement of the incidence of liver tumours in susceptible strains of mouse may require protracted treatment with high doses of a microsomal enzyme inducer.

1.4.2 Carcinogenicity studies in rats

The first chronic feeding study with a microsomal enzyme inducer (DDT) in rats was published by Fitzhugh et al. (1947). A total of 228 animals (Osborne-Mendel Strain) received diets containing technical DDT at concentrations of 0, 100, 200, 400, 600 and 800 mg.kg^{-1}. The mortality in DDT-treated groups was very high and of the initial 192 rats ex-

posed to the compound only 81 survived at least 18 months. After 18-24 months of feeding, four rats were reported to have 'low-grade' hepatic cell carcinomas and eleven rats showed nodular adenomatoid hyperplasia (nodules measuring up to 3 mm). Although no liver tumours were found in control rats, hepatic-cell tumours have been reported to occur spontaneously in 1% of the rats in this colony (Fitzhugh et al., 1947). The authors concluded that 'DDT showed a minimal tendency to cause formation of hepatic cell tumours'.

A second carcinogenicity study with DDT in Osborne-Mendel rats was reported by Radomski et al. (1965) and Deichmann et al. (1970). Thirty males and 30 females were exposed for 24-27 months to either 80 or 200 mg DDT.kg^{-1} diet and compared with two control groups of 30 animals of each sex. Two liver tumours were found in the experiment: one occurred in a control female and the other in a female given 200 mg DDT.kg^{-1} diet. Incidences of other tumours were similar in control and treated rats.

Weisburger & Weisburger (1968) reported an experiment in which a group of 15 male and 15 female Fisher rats were given a dose of 10 mg DDT per rat by stomach tube, 5 times a week starting at weaning. Treatment lasted one year, and survivors were observed for a further 6 months. No liver tumours were found. However, the duration of this study was too short and the number of rats per treatment group too small to warrant any firm conclusion.

Recently Rossi et al. (1977) reported an experiment in which 37 male and 35 female Wistar rats were fed on diets containing 500 mg DDT.kg^{-1} for life-span (Table 8). Thirty-six male and 35 female rats served as controls. Of the animals that survived the time at which the first liver nodule was observed in a DDT-treated female (at 80 weeks), 9 out of 26 treated males (34.6%) and 15 out of 27 females (55.1%) were found to bear liver nodules at death. No liver nodules occurred in controls. There was no evidence of metastases to the lungs or any other organ. The authors classified these lesions as neoplastic nodules even though there was no evidence of invasive properties. Furthermore, the observed nodules occurred - very late in life - in the presence of liver damage including centrilobular necrosis and fatty degeneration. The occurrence of nodular hyperplasia in rats chronically exposed to very high doses of DDT could thus represent a form of regenerative liver growth. Consequently, this study provides no convincing evidence for the tumourigenicity of DDT in rats.

Rossi et al. (1977) also investigated the effects of chronic treatment of Wistar rats with another potent microsomal enzyme inducer, phenobarbital-Na (Table 8). The compound was administered at a concentration of 500 mg/l in the drinking water to 36 male and 34 female seven-week-old Wistar rats for life-span. Twenty-two males and 28 females were still alive when the first liver nodule was reported at about 99 weeks. At the termination of this experiment, when the animals had reached an age of 152 weeks, 13 males and 9 females had developed hepatic nodules. No hepatic nodules were observed in a group of 36 male and 35 female control rats. The incidences of non-hepatic neoplasms were comparable in the test and control groups of rats. The effects of phenobarbitone were thus similar to those observed with DDT.

In a recent study reported by Butler (1978), male inbred Fisher rats were fed on diets containing 1000 mg phenobarbitone-Na.kg^{-1} for 103 weeks. Of 33 treated rats surviving 80 weeks and more, 11 showed foci of nodular hyperplasia in the liver. The foci

were usually small, but one rat killed at 102 weeks had a lesion of 0.75 cm diameter, which compressed the surrounding liver. In no case was evidence of local invasion or metastasis found. Thus this experiment provided no evidence to suggest that phenobarbitone-Na induced neoplasms in rat liver.

The studies by Rossi et al. (1977) and Butler (1978) both failed to establish that phenobarbital-induced liver nodules possessed invasive properties, even though the observation period in one of these studies (Rossi et al., 1977) lasted nearly 3 years. Consequently, it would seem incorrect to classify these lesions as neoplastic growth. As both studies yielded evidence of liver damage in phenobarbital-treated rats, it is not unlikely that the observed liver nodules represent a hyperplastic response to compound-induced liver necrosis.

Several carcinogenicity studies with dieldrin in the rat have been published in the last 15 years (Table 9). Fitzhugh et al. (1964) reported an experiment in which groups of 12 male and 12 female Osborne-Mendel rats were fed on diets containing 0, 0.5, 2, 10, 50, 100 or 150 mg dieldrin.kg^{-1} for two years. Survival rates were decreased at 50 mg dieldrin.kg^{-1} and higher doses and the tumour incidences in these groups have little comparative value. In groups of rats given 0.5, 2 or 10 mg dieldrin.kg^{-1}, the number of tumour-bearing (= all tumours) animals were 8/22, 8/23 and 4/18 at 0.5, 2 and 10 mg dieldrin.kg^{-1}, respectively, compared with 3/17 in the controls (Table 9). In these groups, the survival rate was comparable with that in the controls (75% at 18 months). The authors claimed that there was evidence of 'some general type of effect that increased tumour production, without causing any single type of tumour to predominate'. However, the difference between 20/63 tumour-bearing rats in the treated groups and 3/17 in the controls is not significant ($\chi^2 = 0.71$, $P \geq 0.05$). The authors did not observe any liver tumours in this study.

A subsequent carcinogenicity study performed by Walker et al. (1969) also failed to demonstrate an increased overall tumour incidence in rats exposed to dieldrin (Table 9). Diets containing 0.1, 1.0 and 10.0 mg dieldrin.kg^{-1} were administrated to groups of 25 male and 25 female CFE rats for two years. A group of 45 males and 45 females served as controls. The authors reported that 3 female rats on 10 mg dieldrin.kg^{-1} and one control female rat showed focal proliferation of liver parenchymal cells to form microscopic nodules. Liver tumours were not observed.

Two studies with dieldrin in rats were published recently (National Cancer Institute, 1978a) (Table 9). In the first study, dieldrin was administered to groups of 50 Osborne-Mendel rats of each sex at either a low or a high dieldrin concentration. Time-weighted average doses were 29 (low dose) or 65 mg dieldrin.kg^{-1} diet (high dose). Low-dose rats were treated for 80 weeks, followed by 30-31 weeks of observation. Treatment of high-dose rats was terminated after 59 weeks and followed by an observation period of 51-52 weeks. Matched controls consisted of groups of 10 untreated rats of each sex. Pooled controls consisting of the matched control groups combined with untreated animals from similar bioassays of other chemicals (58 male and 60 female rats) were used for statistical evaluation. All surviving rats were killed at 110-111 weeks. A low incidence of hepatocellular carcinomata was observed with no increased frequency for treated groups over controls (males: one control and one 'high-dose' animal; females: one 'low-dose' and one 'high-

dose' animal).

In the second study (National Cancer Institute, 1978b) groups of 24 Fisher 344 rats of each sex were administered either 0, 2, 10 or 500 mg dieldrin.kg^{-1} diet for 104-105 weeks (Table 9). Survival was not adversely affected by treatment. A variety of neoplasms occurred in control and treated rats, but incidence was not related to treatment. No liver tumours were observed. However, 2 control males (8%) and four males on 50 mg dieldrin .kg^{-1} diet showed evidence of nodular hyperplasia. These lesions were classified as non-neoplastic.

The four studies with dieldrin described above have thus failed to demonstrate tumourigenic effects of the compound on rat liver.

The results of an early study with the α-,β-and γ-stereoisomers of hexachlorocyclohexane (HCH), published by Fitzhugh et al. (1950), indicated that these compounds were not tumourigenic in the rat. However, the validity of this study is questionable. The experimental group sizes were small (10 male and 10 female rats per group) and the survival rates were very poor: mean age was 58 weeks in a group of 40 controls and 33-70 weeks in experimental groups.

Recently, Ito et al. (1975) observed hepatocellular carcinomas in a few Wistar rats fed on a diet containing 1,000 or 1,500 mg α-HCH.kg^{-1} for 72 weeks (Table 10). There was also a high incidence of nodular hyperplasia in these treatment groups. Other isomers of HCH were tested for periods up to 48 weeks, which is far too short to warrant any conclusions on the (non-)tumourigenicity of these compounds in rats.

CONCLUSIONS

Long-term studies in rats with DDT, phenobarbital and dieldrin have provided no evidence of carcinogenicity of these compounds in this species (Fitzhugh et al., 1964; Walker et al., 1969; NCI studies, 1978a, 1978b; Radomski et al., 1965; Deichmann et al., 1970; Rossi et al., 1977; Butler, 1978).

The apparent non-tumourigenicity of these microsomal enzyme inducers in rats has led to the opinion that the tumourigenic effects of these agents on mouse liver constitute species-specific events and may consequently bear no relevance to other mammalian species, including man (van Raalte, 1973). This has resulted in a considerable controversy regarding the use of the laboratory mouse in carcinogenicity testing (Tomatis et al., 1973; Grasso & Crampton, 1972). In the case of α-HCH, however, there are indications that this compound may possess tumourigenic potential in mice (Nagasaki et al., 1971, 1972; Ito et al., 1973) and rats (Ito et al., 1975). Consequently, the contention that the tumourigenic effects of microsomal enzyme inducers in various strains of mouse are species-specific events per se may not be a valid generalization. On the other hand, there can be little doubt that some strains of mouse are highly sensitive to the tumourigenic effects of these compounds.

1.4.3 Carcinogenicity studies in other mammalian species

To date, there are only a few long-term studies with microsomal enzyme inducers in mammalian species other than rats and mice.

Two long-term feeding studies with DDT were conducted in hamsters by Agthe et al. (1970) and Graillot et al. (1975), respectively. The results of these studies provided no evidence of tumourigenicity of DDT in this species.

Agthe et al. (1970) fed groups of 30 male and 30 female Syrian Golden Hamsters on diets containing 500 or 1000 mg DDT.kg^{-1} for 44 weeks. Survivors at 50 weeks were 70/115 treated versus 59/79 control animals. All treated animals and 62/79 controls had died by the 90th week. Eleven treated hamsters developed tumours at different sites (including one liver tumour) as did 8 controls.

Graillot et al. (1975) fed groups of 30 male and 40 female hamsters on diets containing 0, 250, 500 or 1000 mg DDT.kg^{-1} for a period of 78 weeks and observed no lesions which could be attributed to DDT-treatment.

A study with DDT in the dog was published by Lehmann (1965). A total of 22 animals approximately equally divided by sex were fed either 0 (2 dogs), 400 (2 dogs), 2000 (4 dogs) or 3200 (14 dogs) mg DDT.kg^{-1} diet. Only the control dogs, the 2 dogs given 400 mg DDT.kg^{-1} and 2 of the dogs receiving 2000 mg DDT.kg^{-1} survived until they were killed (39-49 months). This study, in which no liver tumours were observed in any of the dogs, was clearly too short to warrant any conclusions on the chronic toxicity of the compound.

Similarly, a chronic study reported by Walker et al. (1969) with dieldrin (daily oral doses of 0, 0.005 and 0.05 mg.kg^{-1} body weight) in dogs was terminated after only two years - no liver tumours were found - and provides no indications on the tumourigenicity of dieldrin in the dog.

In a study reported by Wright et al. (1978) Rhesus monkeys were fed on diets containing 0 mg dieldrin.kg^{-1} (5 animals), 0.01 mg dieldrin.kg^{-1} (4 animals), 0.1 mg dieldrin.kg^{-1} (5 animals), 0.5 mg dieldrin.kg^{-1} (5 animals), 1.0 mg dieldrin.kg^{-1} (4 animals), 1.75 mg dieldrin.kg^{-1} (2 animals) and 5.0 mg dieldrin.kg^{-1} (1 animal) for periods up to 6.5 years. Although at the end of this period no obvious alteration in general structure, colour or texture was observed in the livers of dieldrin-treated animals, this study cannot be regarded as a valid carcinogenicity study in a primate species.

CONCLUSIONS

The results of two chronic feeding studies with DDT in hamsters indicate that this compound is not tumourigenic in this species (Agthe et al., 1970; Graillot et al., 1975).

However, non-rodent studies conducted to date with dieldrin (Walker et al., 1969; Wright et al., 1978) and DDT (Lehmann, 1965) cannot be regarded as valid carcinogenicity studies and warrant no conclusions on the tumourigenicity of these agents in these species.

1.4.4 Epidemiological observations in man

Microsomal enzyme inducers such as phenobarbitone (an anti-convulsant drug used in the treatment of epilepsy), DDT (agricultural pesticide, also successfully applied in anti-malaria programs) and dieldrin (agricultural pesticide) have been in use for more than three decades and retrospective epidemiological studies may thus provide evidence on the hazards of these compounds to man.

The first study on plant workers exposed to DDT was reported by Ortelee (1958). Forty men engaged in the manufacture or formulation of DDT were medically examined. Twenty-eight of the men were under 39 years of age, 7 between 40 and 49, and 5 over 50. The length of exposure at the time of the study was less than 1 year for 2 workers, 1-4 years for 21 workers and 5-8 years for 17 workers. The clinical and laboratory studies conducted (history, physical and neurological examination, blood counts and haemoglobin, sulfobromophtalein, cholinesterase, urinary excretion of DDA) revealed no ill effects attributable to DDT. No evidence of neoplasia was found among the 40 workers at the time of investigation. However, this study cannot, for a variety of reasons, provide evidence on the tumourigenicity of DDT in man. The experimental group was small and the majority of the patients were at an age at which the occurrence of human cancer would not be expected. Furthermore, the observation period was limited to 8 years at maximum, which would seem far too short.

In 1966 a study was made of 35 plant workers with 11-19 years (average 15 years) of exposure to high concentrations of DDT (Laws et al., 1967). The ages of these workers ranged between 30 and 63 years (mean: 43 years). Findings from medical history, physical examination, routine clinical laboratory tests, and chest X-ray film did not reveal ill effects attributable to exposure to DDT. No cancer was reported in any of the workers. A follow-up study on liver function utilizing the same group of men was initiated in 1972 (Laws et al., 1973). By that time the duration of exposure to DDT ranged from 16-25 years with a mean and median of 21 years. No clinical indications of hepatotoxicity, hepatic enlargement or liver dysfunction were observed. The results of serum α-fetoprotein analyses were negative in all 20 of the men for whom the test was performed. This study was also based on a small group of occupationally exposed workers. The fate of workers who had left the industry was not investigated. Liver cancer is relatively rare in the Western world and a study of a small group of occupationally exposed workers does not constitute a sufficiently sound basis for the prediction of safety of the compound in the human situation.

Dieldrin has been manufactured since 1954 in a plant of Shell Nederland Chemie N.V. at Pernis (Rotterdam). Several doctors from the plant industrial medical department have reported on the health condition of a total of 826 workers involved in the handling of dieldrin and other pesticides. The results of their studies indicate that occupational exposures to high concentrations of dieldrin for periods up to 17.5 years did not result in any persistent adverse effect on the health of these workers (Hoogendam et al., 1962; Hoogendam et al., 1965; Jager, 1970; Versteeg & Jager, 1973). In Jager's publication (1970) a group of 233 workers with occupational insecticide exposures of more than four years (4-13.3 years, average 7.6 years) was studied. One hundred and eighty-one workers

were still employed by the firm at the time of the study, and their average age was 41 years (range 22-64). Only 2 deaths had occurred, and one had been caused by stomach cancer. Fifty-two workers who had left the company have been the subject of a subsequent report (Versteeg & Jager, 1973). The average age of this group was 47.4 (range 29-72) years, average occupational exposure was 6.6 years (4.0-12.3) and average time since the end of exposure was 7.4 years (4.5-16). Only one death was recorded, and this had not been caused by cancer.

The results of these studies - so far - indicate that dieldrin causes no liver tumours in occupationally exposed workers. Again, the size of the experimental group is relatively small for a thorough epidemiological study (233 patients) and the occurrence of only one case of liver cancer would result in serious problems of interpretation.

The carcinogenicity of anti-convulsant drugs (including phenobarbital) in man was studied by Clemmesen et al. (1974) in a retrospective investigation conducted on 9,136 patients admitted to the Danish epilepsy centre "Filadelfia" between 1933 and 1962. The patients were treated with phenobarbital (100-300 mg), phenytoin (100-400 mg) or primidone (500-1500 mg) daily. In patients treated for up to 10 years, the incidence at all sites except the liver was the same as or lower than that expected when compared with the incidence of the general population in Denmark. In patients treated for more than 10 years, 3 cases of liver cancer were observed in males, whereas 1.1 were expected, and 1 liver cancer was observed in a female where 0.7 was expected. In males, treated for less than 10 years, 1 liver cancer was observed where 0.4 was expected. Clemmesen et al. (1974) reported that one man with liver cancer had been treated with thorotrast, a known liver-carcinogen, 18 years before death. In patients treated for more than 10 years, tumours of brain and nervous system were observed in 10 males (expected 3.5) and 6 females (expected 2.9).

Schneidermann (1974) reconsidered these results with respect to liver tumours and suggested that the cases of liver cancer might represent an increased incidence, but Clemmesen (1975) reported that 3 out of the 4 liver cancers seen in male patients had previously been treated with thorotrast which is known to induce liver tumours in man (Kiely et al., 1973; Macmahon et al., 1947; Mann et al., 1976 ; Smoron et al., 1972).

CONCLUSIONS

The epidemiological evidence obtained with dieldrin and DDT is not sufficiently strong to indicate safety of these compounds in the human situation (Ortelee, 1958; Laws et al., 1967, 1973; Hoogendam et al., 1962, 1965; Jager, 1970; Versteeg & Jager, 1973).

In contrast, the epidemiological studies conducted by Clemmesen and his associates (1974, 1975) with phenobarbital have established that pharmacological doses of the compound have no adverse effect on human health. The epidemiological evidence obtained with phenobarbital, which was shown to be tumourigenic in mice (Walker et al., 1973; Peraino et al., 1973a; Ponomarkov et al. 1976), supports the contention that 'no toxic effect levels' of xenobiotic inducers in humans exist.

1.5 MECHANISTIC ASPECTS OF LIVER TUMOUR FORMATION BY MICROSOMAL ENZYME INDUCERS

Several mechanisms can be envisaged by which xenobiotic enzyme inducers may exert tumourigenic effects in mammalian liver.

1. Homeostatic mechanisms might gradually be deranged by chronic treatment with xenobiotic inducers. It has been suggested that prolonged exposure to excessive functional demands favours the development of tumours and there is evidence from studies with various organs to support this concept (Becker, 1971). If this were so in the case of microsomal enzyme inducers, one would expect to find signs of autonomy of growth and enzyme production in mammalian liver during protracted treatment with these compounds. Present evidence from several studies indicates that the short-term increases in liver size, DNA synthesis and enzyme activities produced by α-HCH (Schulte-Hermann, 1979), phenobarbital (Crampton et al., 1977), BHT (Crampton et al., 1977) or dieldrin (Wright et al., 1972, 1977) did not increase any further by protracted treatment with these enzyme inducers. Even after 80 weeks, the changes induced by phenobarbital in rat liver were reversible on cessation of treatment and re-induced by phenobarbital to the same extent as by the initial treatment (Crampton et al., 1977).

2. Microsomal enzyme inducers or their metabolites might induce somatic mutations, i.e. alterations in the nucleotide sequence in the DNA genome. Such alterations may result from covalent attacks of ultimate carcinogens on DNA or conceivably from indirect mechanisms involving covalent binding to RNA or specific proteins (Miller, 1970). This concept of chemical carcinogenesis assumes that somatic mutations are primary events in carcinogenesis and, therefore, ultimate carcinogens are considered to be mutagens.

The possibility that dieldrin or one of its metabolic products exerts its tumourigenic action on mouse liver by a direct interaction with DNA has been explored by studying the extent of binding of radioactivity to the liver DNA of rodents exposed to $|^{14}C|$-dieldrin in vivo (Wright et al., 1977). The results of these studies indicated that very small amounts of an unidentified biotransformation product of dieldrin became tightly bound to the liver DNA of the CFE rat, the CF-1 mouse and the LACG mouse (Table 11). The extent of binding correlated with the rate of dieldrin metabolism in these animals (Hutson, 1976); the more rapid the metabolism the greater the binding. Thus, binding was highest in the CFE rat, intermediate in the CF-1 mouse and lowest in the male LACG mouse. However, the CFE rat has been reported to be resistent to the induction of liver tumours by dieldrin (Walker et al., 1969) and consequently, there would seem to be no correlation between the extent of binding to liver DNA and susceptibility to liver tumour formation.

The possibility that in vivo exposure to dieldrin might cause DNA strand breakage has been investigated in the livers of rats and mice. No single strand breakage was detected in the livers of either species after acute exposure to high doses of dieldrin (Wright et al., 1977).

Dieldrin has also been evaluated for mutagenic activity in a variety of test systems. The compound gave negative results in the Salmonella-microsome test system (Bidwell et al., 1975). Dominant lethal assays and host-mediated assays with dieldrin on male CF-1

mice have also yielded negative results (Dean & Doak, 1975). A mutagenic event due to intercalation can be ruled out because of the globular structure of dieldrin. It would thus seem that dieldrin is devoid of mutagenic activity or potential, which makes it unlikely that an interaction between the compound and liver DNA could be responsible for the liver tumours.

3. The induction of hepatic mono-oxygenases by microsomal enzyme inducers could render the liver more susceptible to tumour formation as a result of an increased capability to synthesise proximate or ultimate carcinogenic forms of exogenous or endogenous pre-carcinogens.

In many instances, co-administration of carcinogens with microsomal enzyme inducers has been reported to result in reduced rather than in enhanced carcinogenesis (Kunz et al., 1969; Peraino et al., 1971). These reports suggest that other cellular factors may also influence the generation of carcinogenic reactivity from pre-carcinogens. These factors could include rates and modes of transport to the enzyme(s), levels and affinities of physiological and foreign substrates, e.g. inducer, and activities of enzyme systems which catalyse the subsequent metabolism of the primary products of mono-oxygenase action, e.g. epoxide hydratase, glutathione *S*-epoxide transferase and UDP-glucuronyl transferase. An assessment of the relative contributions of these factors to the overall rate of in vivo activation of pre-carcinogens is very difficult, which may explain some of the contradictory results which have been obtained to date.

The relationship between microsomal enzyme induction and liver tumour formation is a central theme of this thesis and will be subject of further discussion in Chapter 4.

4. The induction of cell replication sensitises liver cells to initiating effects of carcinogenic chemicals. Increased susceptibility of dividing cells to experimentally administered carcinogens appears well documented (Pound & Lawson, 1975; Craddock, 1975; Della Porta & Terracini, 1969). However, the relevance of this mechanism for situations in which no carcinogen is deliberately administered is difficult to assess (Schulte-Hermann, 1979).

5. Microsomal enzyme inducers promote the expression of a pre-existing oncogenic factor in susceptible animal species. The high incidence of 'spontaneous' liver tumours in some strains of mice (Walker et al., 1973; Thorpe & Walker, 1973; Tomatis et al., 1972; Turosov et al., 1973; Tomatis et al., 1974; Peraino et al., 1973a; Ponomarkov & Tomatis, 1976) strongly suggests the existence of such a factor in these animals. Promoting effects by xenobiotic inducers on hepatocarcinogenesis have, in fact, been demonstrated. The administration of phenobarbital to rats previously treated with 2-acetylaminofluorene, a known liver carcinogen, accelerated the appearance of liver tumours and increased the number and growth rate of tumor foci (Peraino et al., 1971, 1973b, 1975, 1977). The promoting effect of phenobarbital on liver tumour formation was confirmed using diethyl-nitrosamine (DENA) as a tumour-initiating agent (Weisburger et al., 1975).

Several other microsomal enzyme inducers including DDT (Peraino et al., 1975), BHT (Peraino et al., 1975), PCB (Kimura et al., 1976) and α-HCH (Schulte-Hermann, 1978)

were also found to promote the formation of liver tumours from previously induced lesions by hepatocarcinogens.

CONCLUSIONS

There is no evidence at present to indicate that microsomal enzyme inducers have mutagenic activity or potential (Wright et al., 1977; Bidwell et al., 1975; Dean & Doak, 1975) and it appears unlikely that an interaction between microsomal enzyme inducers and liver DNA could be responsible for the induction of liver tumours in various strains of mouse.

However, microsomal enzyme inducers, such as phenobarbital, DDT, BHT, PCBs and α-HCH, were found to promote the formation of liver tumours in rats previously treated with liver carcinogens such as 2-AAF or DENA (Peraino et al., 1971, 1973b, 1975, 1977; Weisburger et al., 1975; Kimura et al., 1976 Schulte-Hermann, 1978).

This experimental evidence suggests that the tumourigenic effects of various microsomal enzyme inducers in susceptible strains of mouse could be due to promotion of the expression of pre-existing oncogenic potential in these animals. This hypothesis is supported by the observation that susceptible strains of mouse possess a background incidence of 'spontaneous' liver tumours (see Section 1.4.1).

1.6 RATIONALE OF EXPERIMENTATION

Experimental evidence indicates that the susceptibility of various strains of mouse to the tumourigenic effects of microsomal enzyme inducers could well be related to the presence of pre-existing oncogenic potential in these animals: firstly, susceptible strains of mouse, e.g. CF-1 and C3H mice, show a relatively high incidence of 'spontaneous' liver tumours (see Section 1.4.1) and, secondly, there is evidence to indicate that various microsomal enzyme inducers promote the development of liver tumours in rats previously treated with liver carcinogens such as 2-AAF or DENA (see Section 1.5).

Consequently, the formation of liver tumours in mice exposed to microsomal enzyme inducers could well be due to promotion of the expression of pre-existing oncogenic factor(s) in these animals.

The aim of the present study was to investigate the effects which environmental factors, e.g. the animal diet and bedding, might have on the 'spontaneous' incidence of liver tumours in a tumour-susceptible strain of mouse (CF-1). Both environmental factors are known to contain *naturally occurring* microsomal enzyme inducers (Ferguson, 1966; Vesell, 1967; Loub et al., 1975; Babish & Stoewsand, 1975, 1977). Additionally, commercial diet (CD) may contain traces of carcinogenic agents, e.g. nitrosamines or aflatoxin B_1 (Schoental, 1974) and softwood sawdust (S) is frequently contaminated with 'moth proofing' wood preservatives, e.g. pentachlorophenol, dieldrin, HCB, DDT or endrin (Baldwin, unpublished publications).

One consideration was that if apparently unrelated *xenobiotic* microsomal enzyme inducers could enhance the incidence of liver tumours in various strains of mouse, similar effects might be expected from *naturally occurring* microsomal enzyme inducers in the mouse's environment.

In addition, the presence of carcinogenic substances in the animal's environment might be a major cause of the development of 'spontaneous' liver tumours in tumour-susceptible strains of mouse. The administration of a purified semi-synthetic diet and maintenance on purified bedding, such as shredded filter paper, might, therefore, significantly reduce the *initiation* of liver carcinogenesis in tumour-susceptible strains of mouse. It was decided to study the possible implications of such effects in mice treated with a potent microsomal enzyme inducer (dieldrin).

1.7 SELECTION OF PARAMETERS FOR BIOCHEMICAL INVESTIGATIONS IN MOUSE LIVER

One of the main objects of the present study was to investigate the relationship between microsomal enzyme induction (by naturally occurring agents in diet and bedding and by a xenobiotic inducer) and liver tumour formation in a susceptible strain of mouse (CF-1). The activities of four drug-metabolizing enzyme systems were selected for assay:

1. *Mono-oxygenase* *p*-Nitroanisole *O*-demethylation was used as an index of liver mono-oxygenase activity. As was described in Section 1.3.1, several substrates should ideally be used to achieve proper perspective in the type of mono-oxygenase induction caused by the various experimental factors. However, practical considerations i.e. that such assays could be conducted only at the expense of assays of other drug-metabolizing enzyme systems, imposed limitations on the number of substrates used for the determination of liver mono-oxygenase activity.

2. *Epoxide hydratase* This enzyme catalyses the hydration of epoxides, highly reactive and toxic intermediates which may arise from aromatic hydrocarbons and other substrates by mono-oxygenase action (see Section 1.3.2). Epoxide hydratase may be induced by classical inducers, such as phenobarbitone and 3-MC. Enhancement of the enzyme activity by experimental factors could affect the generation of proximate and ultimate carcinogenic forms of environmental pre-carcinogens. Interaction between epoxide hydratase and dieldrin was considered likely because of the epoxide ring in the dieldrin molecule.

3. *Glutathione S-epoxide transferase* This enzyme system also plays a prominant role in the inactivation of toxic intermediates formed by mono-oxygenase action and may, similar to the activity of epoxide hydratase, affect the formation of proximate and ultimate carcinogens. The enzyme activity is inducible by microsomal enzyme inducers such as phenobarbital (see Section 1.3.2).

4. *UDP-Glucuronyl transferase* This enzyme system is implicated in the metabolism of dieldrin in mammals (Hutson, 1976). In addition, glucuronidation constitutes a major pathway by which the body inactivates a wide variety of lipid-soluble endogenous and exogenous compounds. The activity of these enzymes may, therefore, be relevant to the fate of reactive metabolites formed by mono-oxygenase action. The enzyme(s) are inducible by common microsomal enzyme inducers such as phenobarbital and 3-MC. (see Section 1.3.2).

Information on the type of liver enlargement in the various experimental treatments was provided by *liver DNA* assays. As described in Section 1.2.1, changes in the liver DNA concentration and in the total DNA content of the liver are indicative of the occurrence of hypertrophy or hyperplasia or a combination of the two. In addition, many of the results were expressed on a liver DNA weight basis in an attempt to relate the measurements directly to number of liver cells.

Glucose-6-phosphatase (G-6-Pase) activity was measured in whole liver homogenates and liver microsomes. G-6-Pase being located exclusively in the endoplasmic reticulum of hepatocytes was used as a marker enzyme for the microsomal fraction. The recovery of microsomal protein after subcellular fractionation was based on the retention of G-6-Pase in the microsomal fraction. In addition, depression of G-6-Pase activity has been observed after treatment with a range of liver toxins and liver carcinogens (Feuer et al., 1965). Consequently, G-6-Pase assays also served to monitor toxic effects of experimental factors on hepatocytes.

Similarly, *glutathione* assays were conducted to provide further information on the effects of reactive, i.e. electrophilic, metabolites formed from naturally occurring or synthetic compounds in diet and bedding.

The concentration of *dieldrin* was routinely assayed in livers of dieldrin-treated mice and mice not treated with dieldrin (hereafter referred to as non-dieldrin treated mice). The results of these assays served to indicate to what levels of dieldrin the livers of dieldrin-treated mice had been exposed and also provided a check on the possibility that dieldrin-containing diets might, at some stage, have been erroneously given to non-dieldrin treated mice.

2 Animal experiments, methods and materials

2.1 ANIMAL EXPERIMENTS

2.1.1 Design

The effects of a conventional rodent diet were studied using a purified (semi-synthetic) diet, compounded in this laboratory, as control. Shredded filter paper was used as an alternative bedding to softwood sawdust and dieldrin was chosen as a model xenobiotic microsomal enzyme inducer known to increase the incidence of liver tumours in the CF-1 mouse (Walker et al., 1973; Thorpe & Walker, 1973). Investigation of each of these factors, singly and in combination resulted in 8 experimental treatments (Table 12).

2.1.2 Breeding schedule

The purpose of the schedule was to produce male CF-1 mice that had been continually exposed to specific experimental regimes during both the pre-natal and post-natal periods. Practical considerations, i.e. liver biochemistry in sufficient animals of equal age per treatment group, necessitated a phased breeding schedule: male CF-1 mice were bred and reared during each week of a 6-week period.

Each week over a 6-week period, 32 virgin female CF-1 mice (8 weeks old at a time of allocation) were randomly allocated to eight different treatments (= 4 female mice per treatment). The females were housed individually. Two weeks after allocation to treatments, a randomly chosen virgin male CF-1 mouse of the same age was assigned to each female. The males remained with the females until the presence of vaginal plugs indicated that the females had been mated. Individual females and their offspring were maintained on the specific pre-mating treatments during gestation and pre-weaning phases. After weaning, each male offspring was maintained on the same treatment as his female parent. Males from the same treatment group and of the same age were housed together (up to 5 mice per cage). Parent females and female offspring were discarded. Numbers of male CF-1 mice weaned off in each treatment group are shown in Table 13.

Dieldrin-treated parent females showed signs of hyper-irratability which resulted in an increased occurrence of litter destruction. As a consequence the size of some of the dieldrin treatments was smaller than anticipated. Mice were killed at intervals for biochemical and morphological investigations of their livers.

2.1.3 Maintenance

The animals were housed in plastic cages approximately 30 x 13 x 12 cm with a wire mesh top and a layer of sawdust or shredded filter paper on the bottom. The cages were cleaned twice weekly. The temperature of the animal room was maintained at 20 ± 2°C and both feed and water (local mains supply) were offered ad libitum. Throughout the trial, the animals were inspected daily and killed when moribund.

2.2 METHODS

2.2.1 Preparation of liver homogenates and subcellular fractions

The animals were killed by cervical dislocation. The livers were quickly excised, the gall bladder was removed and the tissue was chilled in ice-cold 0.25 M (isotonic) sucrose solution, pH 7.4, for a few minutes. In livers with macroscopic nodules, the nodular tissue was separated from host liver tissue whenever the size of the lesion(s) would allow all of the biochemical measurements to be carried out (ca. 500 mg tissue). Nodular tissue was then worked up as a separate sample at the expense of one of the control livers. Small nodule(s), comprising not more than a total of a few hundred mg were not separated from the rest of the tissue and results were classified as those obtained in non-nodular liver tissue. The livers and where appropriate, host tissue and nodular tissue, were weighed. Weighed samples of tissue were pressed into a homogenising tube and homogenised in approximately 6-7 ml of ice-cold isotonic sucrose solution, pH 7.4. Fifteen passes of the pestle with a clearance of 0.01 inch (0.252 mm) were used at 1452 rev/min. The final volume of the homogenate was adjusted to 10% w/v by the addition of ice-cold isotonic sucrose (pH 7.4). Ten ml of liver homogenate was fractionated by centrifugation at 11,000 *g* for 15 minutes to remove cell debris, nuclei and mitochondria and subsequently at 200,000 *g* for 40 minutes to obtain the microsomal and soluble fraction. The microsomal pellet was resuspended in 5 ml 0.25 M sucrose solution (pH 7.4).

2.2.2 Enzyme assays

2.2.2.1 p-Nitroanisole O-demethylase

p-Nitroanisole *O*-demethylase activity was assayed to monitor mono-oxygenase activity in accordance with the method described by Netter & Seidel (1964). For the standard assay the reaction mixture, in a total volume of 4.045 ml, contained 50 µmol Tris-chloride buffer (pH 7.4), 19.2 µmol $MgCl_2$, 17.2 µmol disodium glucose-6-phosphate, 1.52 µmol $NADP^+$, 4 units glucose-6-phosphate dehydrogenase (1 unit will oxidise 1 µmol of glucose-6-phosphate to 6-phosphogluconate per minute at pH 7.4 at 25°C in the presence of $NADP^+$), 0.5 ml of microsomal suspension (containing 1.5-3.0 mg of protein), and 8 µmol of *p*-nitroanisole, (added in 25 µl of acetone just prior to incubation). The mixture was shaken at 37°C (water bath) for 10 minutes, in air. The reaction was stopped by the addition of 1 ml of 20% w/v trichloroacetic acid (TCA). Enzyme activity was determined in dupli-

cate and compared with a blank (boiled microsomal suspension). After the addition of TCA, the precipitated protein was removed by centrifugation and 1 ml of 20% w/v Na_2CO_3 was added to the recovered supernatant. *p*-Nitrophenol concentration was estimated by ring the extinction at 420 nm in a Unicam SP 600 spectrophotometer and comparison with a range of standard solutions.

2.2.2.2 Epoxide hydratase (EC. 4.2.1.63)

The activity of this microsomal enzyme was measured as described by Oesch et al.(1971) using |7-^{3}H|-styrene oxide as a substrate. Incubation mixtures in a final volume of 0.4 ml, contained 5 µmol Tris-chloride buffer (pH 8.7 at 37°C), 100 µg Tween 80, 0.2 ml of microsomal suspension (containing 0.5-1.2 mg of protein), and 1.01 µmol |7-^{3}H|-styrene oxide (11 x 10^4 disintegrations per minute, dpm) which was added in 25 µl of acetonitrile just prior to incubation. After incubation at 37°C for 10 minutes, the reaction was terminated by the extraction of the substrate into 10 ml of light petroleum (b.p.40-60°). The organic phase was removed by freezing the aqueous phase in dry ice-acetone and decanting the light petroleum. After thawing, the extraction procedure was repeated and the product, |7-^{3}H|-styrene glycol, was subsequently extracted from the aqueous phase into 2 ml of ethylacetate. Duplicate aliquots (0.2 ml) were counted in 10 ml of NE 260 micellar scintillator solution. Boiled enzyme preparations (5 min, 100°C) served as control.

2.2.2.3 UDP-glucuronyl transferase (EC 2.4.1.17)

The activity was assayed by measuring the rate of glucuronidation of *p*-nitrophenol in the presence of uridine-diphosphoglucuronic acid (UDPGA) and Triton X-100 (octylphenoxypolyethoxyethanol) as described by Pogell & Krismann (1960). The reaction mixture, in a final volume of 0.6 ml, contained 50 µmol of phosphate buffer (pH 7.3), 118 nmol UDPGA, 1.2 µl TX-100, 0.2-0.3 ml of microsomal suspension (containing 0.5-1.5 mg of protein), and 100 nmol *p*-nitrophenol. The incubation mixture was shaken at 37°C for 30 minutes. The reaction was stopped by the addition of 0.1 N TCA (2.4 ml). Enzyme activity was determined in duplicate and compared with a blank which was prepared by omitting UDPGA in the incubation mixture. After the addition of TCA, precipitated protein was removed by centrifugation and 10 N potassium hydroxide (60 µl) was added to the recovered supernatant. The concentration of *p*-nitrophenol was determined by measuring the extinction at 400 nm in a Unicam SP 600 spectrophotometer and comparison with a range of standard solutions.

2.2.2.4 Glutathione S-epoxide transferase (EC 4.4.1.7)

A convenient radiometric assay using styrene oxide as a substrate was described by James et al. (1976). Styrene oxide is known to conjugate spontaneously with glutathione, and therefore the feasibility of this assay was checked under conditions of optimal substrate concentrations. Incubation mixtures contained 100 µmol Tris-chloride buffer (pH

7.4, 37°C), 7.5 µmol reduced glutathione, 0.25 mg soluble fraction protein and water in a total volume of 1.5 ml. The tubes were placed in a shaking water bath at 37°C for a few minutes to attain this temperature. The reaction was started by adding the substrate (styrene oxide) in solution in acetonitrile (50 µl). Three different substrate concentrations were employed: 5 µmol |7-^{3}H|-styrene oxide (5.5×10^5 dpm) per incubation mixture (3.33 mM), 10 µmol (1.1×10^6 dpm) per incubation mixture (6.67 mM), and 25 µmol (2.75×10^6 dpm) per incubation mixture (16.67 mM). Boiled enzyme preparations served as controls. After an incubation time of 5 minutes the reaction was terminated by adding 4 ml ethylacetate and vigorous mixing. Unreacted styrene oxide and any styrene glycol formed (by non-enzymic hydration) were extracted in the organic phase leaving the glutathione conjugate, in this case *S*-(2-hydroxy-1-phenylethyl)glutathione, in the aqueous phase. The ethylacetate layer was removed and the extraction repeated twice. Duplicate aliquots of the aqueous phase (0.5 ml) were subsequently counted in 10 ml NE 260 scintillation mixture.

The results indicated that spontaneous conjugation increased linearly with substrate concentration (Figure 2). The optimal substrate concentration under these assay conditions was in the region of 15 mM. When the highest substrate concentration (16.7 mM) was employed, the spontaneous conjugation accounted for approximately 35% of the total reaction. Spontaneous conjugation occurred exclusively with glutathione. Omission of the soluble protein fraction in the incubation mixture or replacement by an equivalent amount of bovine serum albumin resulted in identical rates of spontaneous conjugation. On the basis of this evaluation it was decided to employ styrene oxide at a concentration of 16 mM for routine assays. For these assays, the soluble fraction was diluted ten times with 0.25 M sucrose (pH 7.4), yielding a protein concentration of approximately 0.5 mg.ml^{-1}.

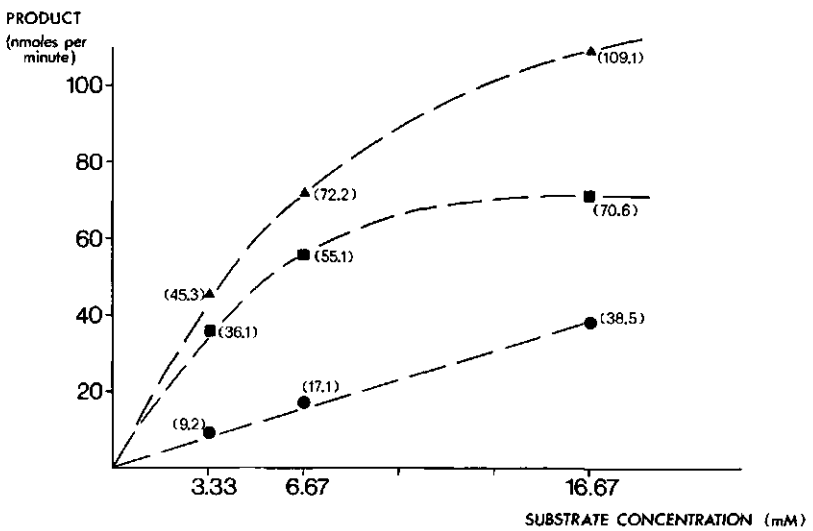


Fig. 2. The effect of substrate concentration on non-enzymic and liver glutathione-*S*-epoxide transferase catalysed conjugation of |7-^{3}H|-styrene oxide with glutathione in vitro. Details of assay conditions are described in the text. ▲ Total conjugation, ■ enzymic conjugation, ● non-enzymic conjugation (boiled enzyme suspension).

2.2.2.5 Glucose-6-phosphatase (EC 3.1.3.9)

G-6-Pase activity was measured in whole liver homogenates and in liver microsomal fractions. The enzyme activity was determined by a modification of the method described by Harper (1965). Samples were diluted with 0.25 M sucrose solution (pH 7.4) and to duplicate portions (0.2 ml) was added a solution of disodium glucose-6-phosphate (0.2 ml; 0.08 M) in 0.1 M citrate buffer, pH 6.5. The mixtures were incubated for 10 minutes at 37°C. Enzymic reaction was stopped by the addition of 10.8% v/v TCA (5 ml). Boiled liver homogenates or liver microsomes served as controls. After the addition of TCA, liberated inorganic phosphate was determined in the protein-free supernatant by a method described by Fiske & Subbarow (1925).

2.2.3 Chemical assays

2.2.3.1 Protein

Homogenised tissue samples and samples of microsomal and soluble fractions were diluted with distilled water to a protein concentration of approximately 100 µg/ml and the protein content was determined (Lowry et al., 1951) by comparison with a series of standard solutions containing 0-200 µg/ml of crystalline bovine plasma albumin.

2.2.3.2 Liver DNA

Homogenised tissue samples (2 ml) were washed three times with 10 ml 0.2 N perchloric acid (PCA) at 0°C. Deoxyribonucleic acid was subsequently extracted in 2 x 3 ml 0.5 N PCA by heating at 70°C for 15 minutes. The nucleic acid extract was adjusted to 10 ml with 0.5 N PCA and deoxyribonucleic acid content was measured colorimetrically with the diphenylamine reaction described by Burton (1956).

2.2.3.3 Reduced glutathione

Homogenised tissue samples (1 ml) were deproteinized by the addition of absolute ethanol (1.9 ml) and 150 mM KH_2PO_4, pH 5.5 (0.1 ml) and subsequent centrifugation (Johnson, 1966). Glutathione content was measured in the protein-free supernatant with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) as described by Beutler et al. (1963). The disadvantage of the DTNB-method is its non-specificity: the reagent also conjugates with thiols other than glutathione. Two independent groups of workers (Crowley et al., 1975; Moron et al., 1977) have compared the results obtained with the DTNB-method with those obtained with a specific enzymatic assay for reduced glutathione based on the use of a glutathione *S*-transferase. The results of these investigations indicate that for tissues such as lung and liver, these two methods give nearly identical results so that the use of the DTNB-method in the present study, seems justifiable.

2.2.3.4 Liver dieldrin concentration

A sample of liver homogenate (1 ml) was stirred with acetone-washed, dried, anhydrous sodium sulphate until the sample was dry. The mixture was extracted with 4 x 25 ml 2 : 1 v/v hexane : acetone over a steam bath. The extract was boiled to almost dryness and the residue was dissolved in approximately 2 ml hexane. Non-polar components in the extract were separated from dieldrin on a column containing 3 g deactivated (with 3% water) florisil. Hexane (25 ml) and 1% acetone/hexane (25 ml) were used as eluents. The latter fraction was analysed by gas-liquid chromatography with electron capture detection for dieldrin (temperature: 190°C; gas: 5% methane, 95% argon; flow rate: 50 ml/min). The recovery of dieldrin was determined by spiking liver homogenate from a control animal with a suitable dieldrin standard (1 ml of 0.5 $\mu g.ml^{-1}$). The recovery efficiency (%) based on 18 separate analyses was 91.0 $\pm$ 6.0 (mean $\pm$ standard deviation, range 79-100%).

2.2.4 Statistics

Results obtained in the various treatment groups were analysed on statistical significance with Student's t-test using mice maintained on SSD and F as controls. This test was also used when data obtained in dieldrin-treated mice were analysed versus data obtained in respective non-dieldrin treated mice.

A comparative statistical analysis of data obtained in non-nodular and nodular liver tissue was performed using Student's paired t-test.

2.3 MATERIALS

2.3.1 Diets

The standard laboratory feed used was Laboratory Animal Diet 2 (LAD 2) supplied by Spillers Ltd., Newmarket, Suffolk, U.K. The chemical composition of this diet, based on data supplied by the manufacturer, is shown in Table 14. Batches of diet were routinely analysed on the presence of aflatoxins (B_1, B_2, C_1 and C_2) by the British Food Manufacturing Industries Research Association, Leatherhead, Surrey, U.K. The results of these analyses were negative throughout the trial. The possibility that other toxins, e.g. nitrosamines, were present in this diet was not investigated. Semi-synthetic diet was compounded in this laboratory on the basis of the 1969 recommendations of the Laboratory Animals Association (LASA) Nutrition Study Group (Dietary standards for laboratory rats and mice, 1969). The composition of this diet is shown in Table 15. Folic acid was not included in the diet since the requirement of this vitamin is satisfied through coprophagy (Hötzel & Barnes, 1966). Potato starch served as an indigestible diet component (Jelnick et al., 1952).

Casein, sucrose and mineral components were supplied by British Drug Houses Chemicals Ltd., Poole, Dorset, U.K. Most vitamins were purchased from Sigma Chemical Company Ltd., London, with the exception of vitamins A, D, E and K which were supplied by Roche Products Ltd., Dunstable, Bedfordshire, U.K. Corn oil (Mazola brand) was purchased locally. Corn starch and potato starch were obtained from Rickards Ltd., Beckingham, Kent, U.K.

2.3.2 Bedding materials

Sawdust bedding material was obtained from W.P. Ushers Ltd., London. This softwood bedding is derived predominantly from Douglas Fir *(Pseudotsuga spp.)* grown in the Scandinavian countries. Samples of sawdust, taken at various time intervals, were analysed (by g.l.c. with electron capture detection) for common chemicals and pesticides used as 'moth proofing' agents in wood preservation (Baldwin, pers. commun.). The results of these analyses indicated that pentachlorophenol was the main contaminant in this type of softwood bedding (concentrations ranging between 0-100 µg/g sawdust). The concentrations of dieldrin, HCB, γ-HCH, β-HCH, endrin, pp'DDE, pp'DDD and pp'DDT were negligible (< 0.1 µg/g).

The second type of bedding material was prepared by shredding Whatman filter paper No. 1 (supplied by Scientific Furnishings Ltd., Chichester, Sussex, U.K.).

2.3.3 Chemicals

|7-^{3}H|-styrene oxide was purchased from the Radiochemical Centre, Amersham, Buckinghamshire, U.K. The compound was prepared by the oxidation of |7-^{3}H|-styrene with m-chloroperoxybenzoic acid, and purified by solvent extraction and fractional destillation (bp 88°C, 26 mm). Radiochemical purity was demonstrated by thin layer chromatography on silica gel GF plates with authentic styrene oxide in four different solvent systems: benzene-chloroform (1 : 1 by vol.); benzene-light petroleum (1 : 1 by vol.); chloroform-light petroleum (1 : 1 by vol.); benzene-ethylacetate-chloroform (1 : 1 : 1 by vol.). After dilution with freshly distilled styrene oxide to a specific activity of 50 µCi/mmd (0.42 µCi/mg), the product was stored at -15°C. *p*-Nitroanisole was obtained from Eastman Organic Chemicals, Rochester, New York. Glucose-6-phosphate (disodiumsalt), NADP, glucose-6-phosphate dehydrogenase (from torula yeast), uridine 5'-diphosphoglucuronic acid (UDPGA), calf thymus DNA, bovine serum albumin, diphenylamine, *p*-nitrophenol, TX-100 (octylphenoxypolyethoxyethanol), Tween 80 (polyoxyethylene sorbitan mono-oleate), 5,5'-dithiobis-(2-nitro-benzoic acid) (DTNB) and reduced glutathione were supplied by Sigma Chemical Company Ltd., London. NE 260 micellar scintillator was purchased from Nuclear Enterprises Ltd., Edinburgh. All reagents and solvents were of A.R. grade.

Dieldrin (1,8,9,10,11,11-hexachloro-4,5-*exo*-epoxy-2,3,7,6,-*endo*-2,1,7,8,-*exo*-tetracyclo 6.2.1.1.^{3,6}0^{2,7} dodec-9-ene) (von Bayer - IUPAC nomenclature), trivially known as HEOD, purity greater than 99%, was supplied by the Agricultural Chemicals Division of Shell Biosciences Laboratory, Sittingbourne, Kent, U.K. Dieldrin was administered to diets in corn oil solution (10 mg in 5 g of corn oil for 1 kg diet). An identical quantity of corn oil was added to control diets. Randomly taken samples of the final diet were routinely analysed (by g.l.c. with electron capture detection) on dieldrin content to insure an equal distribution of the chemical in every batch of diet that was used.

3 Results

3.1 EFFECT ON BODYWEIGHT, LIVER WEIGHT, LIVER DNA AND PROTEIN CONTENT OF LIVER AND SUB-CELLULAR FRACTIONS OF HEPATOCYTES

Oral exposure of CF-1 mice to dieldrin had no effect on bodyweight (Table 16). A significant difference was observed after 68-72 weeks between dieldrin-treated and non-dieldrin treated mice on conventional diet and sawdust bedding, but this was primarily due to relatively high bodyweights in the latter treatment group, of which only two mice were killed. This effect is, therefore, unlikely to be compound-related. Initially, mice fed on conventional diet showed slightly higher bodyweights than those maintained on semi-synthetic diet, but this tendency disappeared with increasing duration of treatment. Bodyweights in all treatment groups increased slightly with the passage of time.

Results obtained with 15-week-old CF-1 mice showed that the administration of 10 mg dieldrin.kg^{-1} diet resulted in pronounced generalized liver enlargement ranging from 37.5% in mice maintained on conventional diet and filter paper bedding to 56.5% in mice maintained on semi-synthetic diet and sawdust bedding (Table 17). Similar effects were seen after 52 weeks of exposure. At this stage, however, generalized liver enlargement in some of the mice exposed to dieldrin was accompanied by the occurrence of advanced nodular liver lesions, which resulted in dramatic increases in relative liver weight (RLW).

Advanced liver nodules were seen in 2/6 mice in each of three dieldrin treatment groups (SSD + S + 10 mg dieldrin.kg^{-1} diet, CD + F + 10 mg dieldrin.kg^{-1} diet, and CD + S + 10 mg dieldrin.kg^{-1} diet), but not in dieldrin-treated mice on semi-synthetic diet and filter paper bedding. However, at subsequent investigations after 68-72 weeks of exposure, liver nodules were observed in all of the four dieldrin-treatment groups. These results indicate that dieldrin exerts tumourigenic effects on mouse liver even when the animals are fed on a purified (semi-synthetic) diet and maintained on uncontaminated (filter paper) bedding, and thus it is unlikely that the compound causes liver tumours by facilitating or exacerbating the action of a potent environmental liver carcinogen.

In the case of non-dieldrin treated mice and using data obtained from mice maintained on semi-synthetic diet and filter paper bedding as the baseline, the administration of conventional diet to mice and exposure to sawdust bedding, both singly and in combination, caused only marginal enlargement of the liver (Table 17). Results obtained in these treatment groups were very similar at the various intervals of exposure. Small nodular lesions were found after 92 weeks in 2/6 mice on semi-synthetic diet and sawdust bedding and in 1/6 animals on conventional diet and sawdust bedding. The occurrence of such lesions caused a very slight increase in mean RLW in these two treatment groups.

Liver enlargement in mice exposed to dieldrin was found to be due to a combination

of hyperplasia (increases in genetic material of the liver) and hypertrophy (increases in the ratio of cytoplasmic to nuclear volume). A decrease in liver DNA concentration, which is indicative of liver cell hypertrophy, was usually observed in mice exposed to dieldrin (Table 18). However, these effects were not always statistically significant (range 0-17.5%), and invariably accounted for less than half of the enlargement of the liver. Consequently, liver cell hyperplasia appeared to play a more prominant role than hypertrophy. This was also clearly demonstrated by the extent to which the total DNA content of the liver (expressed per 100 g of bodyweight) was increased in dieldrin-treated mice (Table 19). After 15 weeks of exposure, these increases ranged from 25.8% in mice maintained on semi-synthetic diet and filter paper bedding to 36.8% in mice fed on a conventional diet and maintained on sawdust bedding. In the case of mice maintained on a conventional diet the increases in liver DNA were nearly proportional to the increases in liver weight. Similar effects were observed at later intervals of exposure.

There was no evidence to indicate that non-nodular liver weight increased as a result of prolonged exposure of mice to dieldrin. Even in mice with advanced nodular liver lesions, total non-nodular liver DNA (mg per 100 g bodyweight) remained constant and was similar to that found in dieldrin-treated mice with no or relatively small nodular liver lesions (Table 20). Total nodular liver DNA (mg per 100 g bodyweight) varied widely as could be expected (Table 20). The concentration of DNA in nodular liver tissue was usually similar to that found in the non-nodular part of the liver (Table 42). A small decrease in nodular liver DNA concentration was observed after 68-72 weeks in dieldrin-treated mice on a conventional diet and filter paper bedding. This observation suggests a slight increase in the average size of nodular hepatocytes in these mice.

In the case of non-dieldrin treated mice and using mice maintained on semi-synthetic diet and filter paper bedding as the baseline, the administration of conventional diet to mice and exposure to sawdust bedding caused no detectable increase in the total liver DNA content after 15 weeks of exposure (Table 19). Total liver DNA content in 52-week-old mice on semi-synthetic diet (SSD + F and SSD + S) was lower than that measured at 15 weeks. As a result the total liver DNA content in 52-week-old mice on conventional diet (CD + F and CD + S) was significantly higher than baseline values (SSD + F). It is unlikely that these results indicate a hyperplasiogenic effect of conventional diet on mouse liver. After 92 weeks of exposure, however, non-dieldrin treated mice on semi-synthetic diet and sawdust bedding and mice on conventional diet and sawdust bedding showed an enhanced total liver DNA content. Both groups of mice were exposed to sawdust, which would seem to implicate this factor in the observed liver cell hyperplasia. As reported earlier, small liver nodules were seen in the above-mentioned treatment groups (2/6 mice from treatment SSD + S and 1/6 mice from treatment CD + S). Total liver DNA content was clearly enhanced in mice bearing liver lesions (SSD + S : 15.9; 18.5 and CD + S : 15.3), but these results fail to explain all of the increases in total liver DNA observed in the two groups of mice. When mice with liver lesions were excluded, total liver DNA was still significantly enhanced (SSD + S : 14.4 $\pm$ 1.1; 4 observations; and CD + S : 14.2 $\pm$ 2.5; 5 observations).

Dieldrin-induced liver enlargement was not accompanied by any obvious changes in the concentration, i.e. per g liver, of total, microsomal or soluble protein (Tables 21-23).

Only a few significant differences between dieldrin and non-dieldrin treated mice were observed.:

1. After 68-72 weeks of exposure, the concentration of liver protein (Table 21) was significantly reduced ($p < 0.01$) in two dieldrin treatment groups (SSD + 10 mg dieldrin.kg^{-1} diet + S; CD + 10 mg dieldrin.kg^{-1} diet + S). However, at this stage of the experiment, the number of mice in the non-dieldrin control groups were three and two, respectively. These few mice exhibited relatively high liver protein concentrations and it is, therefore, unlikely that the observed effects were compound-related;
2. Fifty-two week-old dieldrin-treated mice on conventional diet and filter paper bedding showed a significantly increased concentration of liver microsomal protein (Table 22). Similar effects were not observed, however, at other intervals of exposure and the significance of the observed effect is not very clear;
3. After 68-72 weeks of exposure, dieldrin-treated mice on conventional diet and filter paper bedding showed significant decreases in the concentration of liver soluble protein (Table 23). However, the corresponding non-dieldrin treatment consisted of only two mice which showed exceptionally high concentrations of liver soluble protein and the observed effect is not, therefore, likely to be related to dieldrin-treatment.

Total liver protein/liver DNA quotients (Table 24), which reflect the protein concentration per liver cell, were significantly enhanced after 15 weeks of exposure in 3 out of 4 dieldrin treatment groups (SSD + F + 10 mg dieldrin.kg^{-1} diet, SSD + S + 10 mg dieldrin.kg^{-1} diet and CD + S + 10 mg dieldrin.kg^{-1} diet). These results confirm the occurrence of hypertrophy in livers of dieldrin-treated mice. However, no such effects were observed at later exposure intervals indicating that liver enlargement at these stages was probably due nearly exclusively to hyperplasia. Liver microsomal protein/liver DNA quotients tended to be slightly higher in dieldrin-treated mice than in the non-dieldrin treated controls (Table 25), but these effects were often not significant. The results suggest only a limited degree of proliferation of SER in livers of dieldrin-treated mice. Similarly, liver soluble protein/liver DNA quotients were slightly enhanced in dieldrin-treated mice (Table 26). This effect was more pronounced after 15 weeks than at later intervals of exposure.

No particular cellular characteristics were detected that were unequaly associated with nodular lesions: liver protein/liver DNA, liver microsomal protein/liver DNA and liver soluble protein/liver DNA quotients were similar to those observed in respective non-nodular liver tissue (Tables 46-48).

The results discussed in this section indicate that exposure of CF-1 mice to dieldrin results in pronounced, generalized liver enlargement most of which is due to hyperplasia. This was evinced by increases in the total DNA content of the liver in dieldrin-treated mice, which were frequently proportional to the increases in liver weight. Liver cell hypertrophy was shown to play a less prominent role in dieldrin-induced liver enlargement. Evidence for the occurrence of liver hypertrophy was provided by decreases in the concentration of liver DNA, and by increases in total liver protein/liver DNA, liver microsomal protein/liver DNA and liver soluble protein/liver DNA quotients in dieldrin-treated mice.

In contrast to the effects of dieldrin, conventional diet and sawdust caused only marginal enlargement of the liver. No hyperplasia was detectable at most exposure intervals and any liver enlargement induced by these factors would consequently seem to be due to hypertrophy. Late in life (after 92 weeks of exposure), however, there was evidence of liver cell hyperplasia in mice exposed to sawdust. The enlargement of the liver in mice exposed to 10 mg dieldrin.kg^{-1} diet was followed by the occurrence of nodular lesions, which were first observed after 52 weeks. The advanced nature of the nodules suggested that they were macroscopically visible well before the animals were one year old. Liver nodules eventually occurred in all of the four dieldrin-treatments. No cellular characteristics were observed that were specifically associated with nodular lesions. Total protein/liver DNA, liver microsomal protein/liver DNA and liver soluble protein/liver DNA quotients in nodules were similar to those observed in respective non-nodular tissue.

3.2 EFFECT ON HEPATOCELLULAR DRUG-METABOLISING ENZYMES

Liver enlargement induced by dieldrin was accompanied by the induction of drug-metabolising enzymes, e.g. *p*-nitroanisole-*O*-demethylase (used as an index of mono-oxygenase activity), epoxide hydratase, glutathione *S*-epoxide transferase and UDP-glucuronyl transferase (Tables 27-30). Conventional diet and sawdust bedding also caused induction of the liver mono-oxygenase system although these effects were less pronounced than that produced by dieldrin. Other drug-metabolising enzymes were only very slightly increased by conventional diet and sawdust bedding (Tables 28-30).

After 15 weeks of exposure the mean percentage increase in *p*-nitroanisole *O*-demethylase activity (Table 27) due to the inclusion of dieldrin in the diet was similar in three of the four dieldrin treatment groups (SSD + 10 mg dieldrin.kg^{-1} diet + S; CD + 10 mg dieldrin.kg^{-1} diet + F; CD + 10 mg dieldrin.kg^{-1} diet + S) ranging from 130% to 205%. In the fourth dieldrin treatment group (SSD + 10 mg dieldrin.kg^{-1} diet + F), the activity of *p*-nitroanisole *O*-demethylase was increased by an average of 660% over its respective control level. This massive increase was a consequence of the low activity exhibited by the respective control animals. In non-dieldrin treated mice and using animals maintained on semi-synthetic diet and filter paper bedding as the baseline, exposure to sawdust bedding resulted in a 267% increase in enzyme activity; conventional diet induced the enzyme approximately one-fold (98%). In combination, these factors caused a 133% increase of *p*-nitroanisole *O*-demethylase activity. Thus the inducing effects of these factors were not additive. Results obtained at later intervals of exposure were similar to those obtained at 15 weeks.

After 15 weeks, a significant increase in liver epoxide hydratase activity (Table 28) was observed in three out of four dieldrin treatments (SSD + 10 mg dieldrin.kg^{-1} diet + F, CD + 10 mg dieldrin.kg^{-1} diet + F and CD + 10 mg dieldrin.kg^{-1} diet + S) ranging from 78-87%, but no evidence for induction was found in dieldrin-treated mice fed on a semi-synthetic diet and maintained on a sawdust bedding. Dieldrin would seem to be only a moderate inducer of this enzyme activity in the livers of mice. Other environmental factors did not exert any significant effect on the activity of hepatic epoxide hydratase. However, after 52 weeks of exposure, small increases in enzyme activity were observed as

a result of feeding a conventional diet and/or exposure of mice to sawdust. The increases produced by these factors were 37% as a result of exposure to sawdust, 115% as a result of feeding a conventional diet and 72% by the combination of the two factors, which suggests that the effects were not additive. The induction produced by the administration of dieldrin was more pronounced than that caused by diet and/or bedding and ranged from 46.4% to 179% over the respective non-dieldrin treated control levels.

The activity of glutathione *S*-epoxide transferase (Table 29), which is located in the soluble fraction of hepatocytes, was induced to 70-120% over control levels in 15-week-old mice exposed to 10 mg dieldrin.kg^{-1} diet. Relatively small increases of enzyme activity were induced by sawdust bedding (37.7%), conventional diet (18.5%) or a combination of the two (28.1%). The latter result suggests that the effects of diet and bedding were non-additive. No evidence for the induction of this enzyme system by diet and bedding was found at 52 weeks, but at 92 weeks non-dieldrin treated mice on conventional diet showed slightly higher activities than non-dieldrin treated mice on semi-synthetic diet (difference ca. 12%). The extent of induction of glutathione *S*-epoxide transferase as a result of dieldrin administration remained constant throughout the observation period.

Fifteen-week-old CF-1 mice fed on diets containing 10 mg dieldrin.kg^{-1} showed an increased activity of liver UDP-glucuronyl transferase (Table 30). The increases were not very pronounced, however, and ranged from 17-48%. Diet and bedding did not influence this enzyme activity. The effect of dieldrin after longer intervals of exposure were very similar to that observed at 15 weeks.

Expression of the various drug-metabolising enzyme activities per unit liver DNA (Tables 31-34), which reflects the activity per cell, yielded a pattern of results that was similar to the observed specific enzyme activities, i.e. activity expressed in terms of unit weight of microsomal or soluble protein (Tables 27-30).

Comparative investigations in non-nodular and nodular liver tissue from the same animals revealed that mono-oxygenase, epoxide hydratase and glutathione *S*-epoxide transferase activities were slightly, but consistently higher in nodular liver tissue (Tables 49-51 and 53-55). No significant differences were seen with respect to UDP-glucuronyl transferase activity (Tables 52, 56).

3.3 EFFECT ON LIVER GLUCOSE-6-PHOSPHATASE ACTIVITY AND LIVER GLUTATHIONE CONCENTRATION

When G-6-Pase activity was expressed in terms of unit weight of microsomal protein, there was a consistent trend towards a slight depression in the activity of this enzyme in dieldrin-treated mice (Table 35). However, when G-6-Pase activity was expressed per mg DNA, which reflects the enzyme concentration per cell, no consistent effect was observed (Table 36). These results suggest that dieldrin-exposure does not cause any depression of hepatocellular G-6-Pase in male CF-1 mice and that the observed trend towards decrease in the specific activity of this enzyme, i.e. activity expressed in terms of unit weight of microsomal protein, probably reflects the occurrence of liver cell hypertrophy in dieldrin-treated mice. Conventional diet and sawdust bedding had no effect on liver G-6-Pase activity.

In contrast to these results, a marked depression of G-6-Pase activity was observed

in nodular liver tissue (Tables 57, 58). G-6-Pase specific activity was reduced to approximately 40-50% of the levels found in non-nodular liver tissue of the same animals (Table 57). Similar effects were observed when the enzyme activity was expressed per unit liver DNA (Table 58). This indicates that nodular hepatocytes are severely G-6-Pase deficient.

The administration of dieldrin to mice had no effect on liver glutathione concentration (Tables 37 and 38). Similarly, neither the feeding of conventional diet nor exposure to sawdust bedding resulted in any change in the concentration of this tripeptide. However, nodular liver tissue was found to be severely glutathione-deficient (approximately 60% of levels found in non-nodular liver tissue) as shown in Tables 59 and 60.

3.4 RESIDUE LEVELS OF DIELDRIN IN THE LIVER

After 15 weeks of exposure, mean concentrations of dieldrin in the liver of mice in the four dieldrin treatment groups ranged from 11.0 to 15.0 $\mu g.g^{-1}$ liver (Tables 39 and 40). Similar levels were found in non-nodular livers of dieldrin-treated mice after 52 weeks of exposure. The concentration of dieldrin in nodular livers was lower (Tables 39 and 40) than the levels found in non-nodular livers of dieldrin-treated mice, but total liver dieldrin content remained unchanged (Table 41). This result suggests that the observed decrease in dieldrin concentration in nodular livers is due to increases in organ weight. No significant differences were observed between the concentration of dieldrin in nodular and non-nodular liver tissue of the same animal (Tables 61 and 62). No dieldrin could be detected in the livers of non-dieldrin treated mice (Tables 58 and 59).

3.5 EFFECT OF DISCONTINUING THE EXPOSURE TO DIELDRIN

The reversibility of dieldrin-mediated changes in nodular and non-nodular liver tissue was studied in CF-1 mice maintained on conventional diet (+ 10 mg dieldrin.kg^{-1}) and filter paper bedding. After 85 weeks of exposure, some remaining mice in this treatment were placed on dieldrin-free conventional diet. After 10 weeks, four of these mice were killed for biochemical investigations of their liver. The results were compared with those obtained in non-regressed mice and also with those obtained in mice maintained on control conventional diet and filter paper bedding.

Very large liver tumours were observed in dieldrin-treated mice after 95 weeks (Table 63) and separation of nodular liver tissue from non-nodular tissue was extremely difficult: most of the liver was affected by advanced lesions. Relative liver weights ranged from 12.63 to 22.47 (mean 17.86 $\pm$ 4.10) and were similar to those found in mice from the same treatment group after 68-72 weeks of exposure (Table 63). Total liver weight was significantly reduced ($P < 0.05$) in regressed mice (Table 63). All of these four mice showed nodular liver lesions, but in a less advanced state which facilitated the separation of nodular tissue from non-nodular tissue. No dieldrin residues were detected in either non-nodular or nodular liver tissues (Table 63). Total liver weights (Table 63) were remarkably similar in regressed mice (4.26 $\pm$ 0.08, RLW: 10.36 $\pm$ 0.23) as well as the proportions of non-nodular liver weight (2.80 $\pm$ 0.17, RLW: 6.80 $\pm$ 0.37) and nodular liver weight (1.46 $\pm$ 0.22, RLW: 3.56 $\pm$ 0.57). Relative non-nodular liver weights in regressed

mice were approximately 55% higher than values observed in non-dieldrin treated controls. This difference is similar to that observed between dieldrin and non-dieldrin treated mice at various intervals of exposure (Table 17), which suggests that no change occurred in the size of non-nodular liver tissue during regression. Consequently, it would seem that the observed reduction of total liver weight in regressed mice was due to changes (decreases) in the size of liver nodules.

An accurate assessment of total non-nodular liver DNA in 95-week-old dieldrin-treated mice was prevented by the occurrence of advanced nodular lesions in many parts of the liver. Nevertheless, results obtained did not deviate significantly from those obtained at earlier intervals of exposure, e.g. after 68-72 weeks (Table 64). In regressed mice, total non-nodular liver DNA was similar to that observed in 68-72 and 95-week-old dieldrin-treated mice (Table 64). This similarity indicates that hyperplasia persisted in non-nodular liver tissue throughout the regression period. Total nodular liver DNA in regressed mice, however, was reduced relative to values observed in dieldrin-treated mice (Table 64). Thus the observed reduction in the size of liver nodules was at least in part due to a reduction in the number of cells. The observation that the concentration of DNA in nodular liver tissue of regressed mice was similar to that observed in nodular liver tissue of dieldrin-treated mice, suggests that no reduction in the size of nodular liver cells occurred during regression and, consequently, it would seem that a reduction in the size of liver cells did not contribute to the observed reduction in the size of liver nodules. However, liver DNA concentration in non-nodular liver tissue of regressed mice was significantly increased ($P < 0.05$) over values observed in non-nodular liver tissue of dieldrin-treated mice (Table 64), which suggests that the average size of non-nodular liver cells decreased during regression. This finding is supported by the observation that non-nodular liver protein/liver DNA quotients were significantly lower ($P < 0.05$) in regressed mice than in dieldrin-treated mice (Table 65). Liver microsomal protein/liver DNA quotients in non-nodular tissues were also slightly, but significantly reduced in regressed mice (Table 65). Thus, in contrast to liver cell hyperplasia, liver cell hypertrophy appears to be reversible in non-nodular liver tissue of mice exposed to dieldrin.

The withdrawal of dieldrin from the diet and subsequent metabolic elimination of the compound resulted in complete regression of the specific activity of the microsomal mono-oxygenase system in non-nodular liver tissue (Table 66). Regression also occurred with respect to epoxide hydratase, glutathione *S*-epoxide transferase and UDP-glucuronyl transferase (Table 66), but the specific activity of these enzymes remained significantly higher than those observed in non-dieldrin treated controls ($P < 0.01$). However, when the enzyme activities were expressed per unit non-nodular liver DNA, which reflects the concentration of the enzyme(s) per liver cell, regression was virtually complete with respect to all of these drug-metabolizing enzyme systems (Table 67). In fact, mono-oxygenase and UDP-glucuronyl transferase activity per unit liver DNA were significantly lower ($P < 0.01$) than values observed in non-dieldrin treated controls (Table 67).

Regression of enzyme activity was also observed in nodular liver tissues but control rates were not attained even though no dieldrin could be detected in these tissues (Table 67).

These results indicate that the changes in drug-metabolizing enzyme activities induced by dieldrin are reversible in both non-nodular and nodular liver tissue. However,

regression was less complete in nodular liver tissue.

G-6-Pase-activity was normal in non-nodular liver tissue of regressed mice (Tables 66 and 67), but remained severely depressed in nodular liver tissue (Tables 66 and 67). In dieldrin-treated mice, a reduction of G-6-Pase activity was also observed in non-nodular liver tissue. It should be kept in mind, however, that separation of non-nodular tissue from nodular liver tissue was extremely difficult in these mice and it is likely that non-nodular liver tissue contained G-6-Pase deficient nodular liver cells.

3.6 EFFECT ON SURVIVAL AND INCIDENCE OF LIVER TUMOURS (by courtesy of Miss K.M. Dix)

3.6.1 Survival

Survival in each treatment group is shown in Figure 3. The data were adjusted for interim kills. If there were n mice initially in a treatment group and d_1 animals had died before any animals had been sacrificed, percentage survival S_1 was calculated as:

$$S_1 = \frac{100\ (n - d_1)}{n}$$

Percentage survival after k animals had been killed and d_2 animals had died subsequently was calculated as:

$$S_2 = \frac{100\ (n - d_1)}{n} \times \frac{(n - d_1) - k - d_2}{(n - d_1) - k}$$

Example: if $n = 100$
$d_1 = 20$
$d_2 = 10$
$k = 30$

then $S_1 = \frac{100\ (100 - 20)}{100} = 80\%$

and $S_2 = \frac{100\ (100 - 20)}{100} \times \frac{(100 - 20) - 30 - 10}{(100 - 20) - 30} = 80 \times \frac{40}{50} = 64\%$

The principal effect observed was a significant reduction of survival rates of mice exposed to dieldrin (Figure 3). Figure 4, which shows pooled survival rates of the four dieldrin treatments and of the four non-dieldrin treatment groups, also serves to demonstrate this effect. The first liver tumour incidence assessment was carried out after 65 weeks of exposure. As shown in Figure 3, survival rates at this stage were > 80% in all of the eight treatment groups, even though this investigation revealed that 70% or more of the mice exposed to dieldrin had nodular liver lesions, many of which showed massive proportions. Bearing in mind that the first liver tumour was observed in a 43-week-old dieldrin-treated mouse, it is surprising that survival in the four dieldrin-

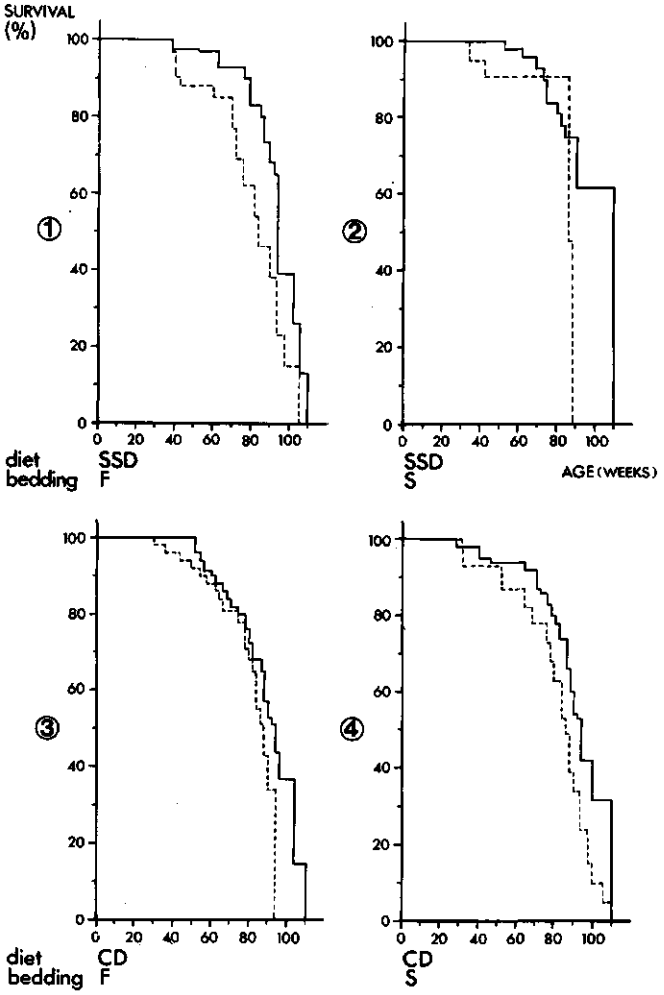


Fig. 3. Survival (adjusted for scheduled kills) against time. The experiment was terminated after 110 weeks. —— 0 mg dieldrin.kg^{-1} diet, --- 10 mg dieldrin.kg^{-1} diet.

treatment groups was still > 60% after 80 weeks of exposure. Fifty percent of the animals receiving 10 mg dieldrin.kg^{-1} diet had died by week 86 (ca. 20 months). Fifty percent survival in non-dieldrin treated mice was reached after 94 weeks (ca. 22 months). On average, 1/10 dieldrin-treated mice survived 100 weeks (ca. 23 months) compared with 4/10 non-dieldrin treated mice.

3.6.2 Liver tumour incidence

Liver nodules were classed according to Walker et al. (1973) as type A: nodular growth of solid cords of parenchymal cells classified as benign tumours, and type B: papilloform and adenoid growth with cells proliferating in confluent sheets with necrosis and increased mitoses (Figure 5). Mice with type B liver tumours often had type A growths

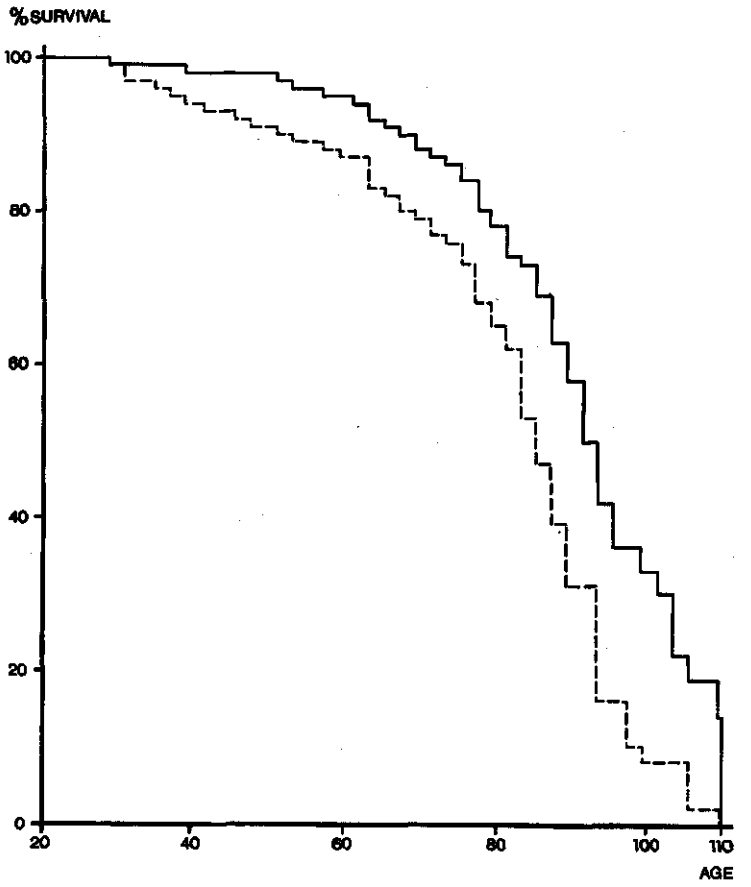


Fig. 4. Pooled survival rates of dieldrin and non-dieldrin treatments. The experiment was terminated after 110 weeks. —— non-dieldrin treatments, --- dieldrin treatments (10 mg.kg^{-1} diet).

as well, but these latter lesions are not included in Column A in the table summarizing liver tumour incidence.

The incidence of liver tumours (Table 68) was analysed for five periods of the study:

1. up to week 65,
2. interim kill at week 65,
3. from week 65 to week 92,
4. interim kill at week 92,
5. from week 92 until end of the study.

For each of these periods and for each of the liver tumour classifications (type A, type B, total) the incidence in groups 2-8 was tested against group 1 (mice maintained on semi-synthetic diet and filter paper bedding) using Fisher's exact test (Bradley, 1968). Significances at the 5%, 1% and 0.1% level have been marked accordingly.

As described above, dieldrin administration to mice resulted in the relatively early

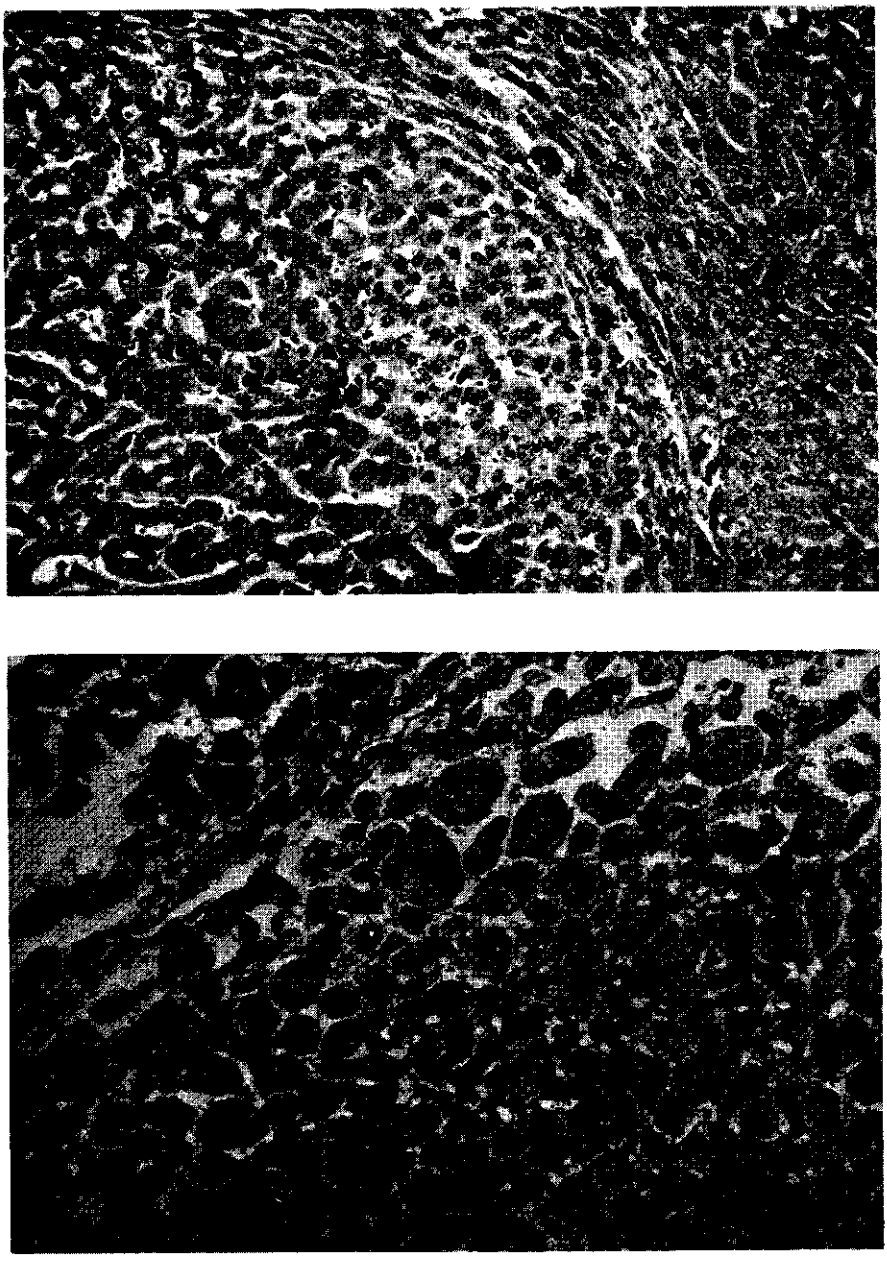


Fig. 5. Classification of liver tumours according to Walker et al. (1973). Type 'A' (above) liver tumour: nodular growth of solid cords of parenchymal cells. Type 'B' (below) liver tumour: papilloform and adenoid growth with cells proliferating in confluent sheets with necrosis and increased mitoses.

appearance of nodular hepatic lesions, the first being observed in a mouse aged approximately 43 weeks. Longer-term exposure to dieldrin resulted in the development of hepatocellular carcinomata. Forty-eight percent of mice with hepatocellular carcinomata ('B' type tumours) that died or were killed between 18 months and the termination of the study showed lung metastases (Table 69).

Liver nodules also occurred in some mice from the groups not treated with dieldrin. However, these nodules were smaller than those observed in the dieldrin groups being usually less than 10 mm in diameter. Two nodules from the non-dieldrin groups showed morphological characteristics of hepatocellular carcinomata. No lung metastases were observed in non-dieldrin treated mice bearing liver nodules (Table 69).

Very few nodules were present in the livers of mice in the non-dieldrin groups at 65 weeks (Table 68). However, the incidence in the corresponding dieldrin-treatment groups was very high, even in animals maintained on a purified diet and filter paper bedding. At 92 weeks, there was some variation in the incidence of nodular lesions in the non-dieldrin treatment groups. Mice on semi-synthetic diet and sawdust bedding showed a significantly increased incidence of liver tumours. The relevance of this effect is difficult to assess in the light of other contrasts between filter paper and sawdust treatments in the absence of dieldrin, e.g. treatment CD + F versus treatment CD + S. Thus, conventional diet and sawdust bedding did not exert any obvious influence on the development of 'spontaneous' tumours in the livers of male CF-1 mice (Table 68). It would appear that, even though conventional diet and sawdust contained agents that induced the mouse liver microsomal mono-oxygenase system, these agents have no intrinsic tumour-promoting activity or are present at concentrations below the threshold for overt tumour-promoting action.

4 Discussion

Several mechanisms can be envisaged by which microsomal enzyme inducers, such as dieldrin, phenobarbitone, DDT, and α, β and γ stereoisomers of HCH, may exert their tumourigenic action in livers of susceptible animal species.

The tumourigenic action of these compounds could be mediated through direct interaction of reactive metabolites with cellular DNA leading to somatic mutations, i.e. alterations in the nucleotide sequence of the DNA genome. This concept assumes that somatic mutations are primary events in carcinogenesis. However, there is at present no evidence to indicate that microsomal enzyme inducers such as dieldrin or phenobarbitone possess mutagenic activity and/or potential (Wright et al., 1977) and it is considered unlikely that these compounds are intrinsically carcinogenic by a direct genotoxic mechanism.

An alternative mechanism could be that microsomal enzyme inducers exert their tumourigenic action in rodent liver by facilitating the action of a potent environmental carcinogen. A possible mechanism of action could be that the induction of liver mono-oxygenases by microsomal enzyme inducers would render the liver more susceptible to tumour formation. The microsomal mono-oxygenase system has been implicated as a key factor in the generation of carcinogenic reactivity from pre-carcinogens (Miller, 1970). This link has led to the opinion that an increased capacity of the microsomal mono-oxygenase system is associated with increases in the rates of generation of ultimate carcinogens formed from environmental pre-carcinogens.

One of the main objectives of this study was to ascertain whether microsomal enzyme inducers were present in conventional diet and sawdust bedding and to establish what effects such naturally occurring agents may have on the incidence of liver tumours in CF-1 mice. The administration of 10 mg dieldrin.kg^{-1} diet to some of the experimental groups served as a positive control, i.e. a potent microsomal enzyme inducer with tumourigenic properties in CF-1 mice.

The results of the investigations showed that exposure of mice to dieldrin caused a 3-4 fold increase in the activity of *p*-nitroanisole *O*-demethylase activity - used as an index of mono-oxygenase activity - and a 2-fold increase in the activities of epoxide hydratase and glutathione *S*-epoxide transferase. UDP-glucuronyl transferase activity was increased approximately 1.5-fold in livers of dieldrin-treated mice.

In the case of non-dieldrin treated mice and using data obtained in mice maintained on semi-synthetic diet and filter paper bedding as the baseline, the administration of conventional diet to mice and exposure to sawdust bedding, both singly and in combination, caused induction of the liver mono-oxygenase system, although this effect was less pronounced than that produced by dieldrin. Other drug-metabolizing enzymes were not or only very slightly increased by conventional diet and sawdust bedding.

A high incidence of liver tumours was observed in dieldrin treated mice after 65

weeks of exposure, even in mice maintained on a semi-synthetic diet and filter paper bedding. In contrast to the effects of dieldrin, conventional diet and sawdust bedding failed to enhance the incidence of liver tumours in CF-1 mice. The observation that conventional diet and sawdust bedding induce the mono-oxygenase system of mouse liver but fail to enhance the incidence of liver tumours indicates that microsomal mono-oxygenase induction is not invariably associated with enhanced liver tumour formation.

The hypothesis that activation of the microsomal mono-oxygenase system is associated with an increase in the generation of carcinogenic reactivity from precursor carcinogens is supported by a positive relationship between the rate of mono-oxygenase catalysed metabolism of pre-carcinogens and the mutation rates in bacterial test systems (Czygan et al., 1973; Bartsch et al., 1975). However, contradictory results have been obtained in animal carcinogenicity tests. For example, the *simultaneous* application of diethylnitrosamine (DENA) and phenobarbital led to a significant reduction in the frequency of liver tumours and to a prolongation of tumour manifestation times (Kunz et al., 1967, 1969). Identical results were reported using different inducers of the mono-oxygenase system, such as 3-MC (Hoch-Ligeti et al., 1968), or PCB (Makiuara et al., 1974).

In contrast, the combination of DENA with halothane or methoxyfluorane, both of which do not induce but rather inhibit the mono-oxygenase system under the experimental conditions used, markedly enhanced the development of hepatocellular carcinomas, while simultaneously lowering the number of hemangioendotheliomas (Kunz, 1969).

The results of in vitro and in vivo experiments may be reconciled by observations by Kunz et al. (1978). This group showed that at low concentrations of a pre-carcinogen substrate (dimethylnitrosamine), phenobarbital pre-treatment decreased, whereas SKF-525A increased formaldehyde production and alkylation intensity (covalent binding to proteins). This situation reflects the in vivo situation during animal carcinogenicity experiments. In contrast, with high substrate (carcinogen) concentrations, phenobarbital pre-treatment increased both formaldehyde production and alkylation intensity, whereas SKF-525A had the contrary effect. Microbial mutagenicity tests can be carried out with non-rate-limiting substrate concentrations, which explains the enhancing effects of microsomal enzyme inducers on the mutation rate of carcinogens in such tests.

The level of possible human exposure to nitrosamines remains substantially below the lowest concentrations used in animal experiments. However, induction or inhibition of microsomal mono-oxygenase following drug administration may also occur in humans. As both the resulting increase and decrease of nitrosamine-mediated alkylation were experimentally found to be increasingly effective the lower the concentration of nitrosamine, and as the level of possible human exposure is even lower than the lowest concentration used experimentally, it has been argued by Kunz et al. (1978) that drug-mediated toxification or detoxification of the carcinogen in this critical dose range might be of considerable importance for humans as well. A major uncertainty about the relevance of animal experiments to the human situation is the finding that all modifying effects of drugs on mono-oxygenase activity are highly dependent on sex and species of the animals used and, in this respect, only little is known about the properties of human mono-oxygenase.

In addition to substrate concentration, number of active centres and rate constants,

numerous other factors can influence the rates of oxygenation of substrates by the liver microsomal mono-oxygenase system in vivo. These include rates and modes of transport to the enzyme, levels and affinities of physiological and foreign substrates, e.g. inducer, and activities of enzyme systems which catalyse the subsequent metabolism of the primary products of mono-oxygenase action, e.g. epoxide hydratase, UDP-glucuronyl transferase and glutathione-*S*-epoxide transferase. Because of the difficulties in assessing the relative contributions of these factors in determining the overall rate of in vivo oxygenation of a given substrate, in vivo rate measurements are required to provide perspective to in vitro determined rate measurements.

In the current study, liver tumours were observed in a few animals maintained on semi-synthetic diet (SSD) and filter paper bedding (F). This confirms the presence of a pre-existing oncogenic factor or susceptibility in this strain of mouse. Although mice maintained on SSD and F may not have lived in an entirely carcinogen-free environment, it would appear unlikely that 'spontaneous' liver tumours in these animals were caused by environmental factors. It is postulated that these tumours are the expression of a pre-existing factor which is genetically linked and possibly viral in origin. Experimental evidence indicates that lymphoid leukosis, lymphosarcomas and leukemias in mice, rats and cats, mammary gland carcinomas in mice, certain sarcomas in mice and other tumours are caused by RNA oncogenic viruses (Gross, 1974, 1978). In many cases, these oncogenic viruses are transmitted from one generation to another ('vertical transmission' as opposed to 'horizontal transmission' of contagious pathogenic agents which spread rapidly from one host to another within the same generation). RNA oncogenic viruses contain a DNA polymerase (reverse transcriptase) which catalyses the synthesis of a DNA copy of the RNA genome (Bauer, 1978). This viral DNA becomes a provirus, that is, it establishes permanent covalent bonds with the cellular DNA. The demonstration that viral DNA is integrated in the cells, in conjuction with the finding that the provirus is transcribed into messenger RNA hundreds of generations after the establishment of a transformed clone suggests a continuing role of viral gene functions in determining transformation (Bauer, 1978).

Virus-like particles have been observed in 'spontaneous' and phenobarbitone-induced liver tumours in C3H mice (Jones & Butler, 1975) but its role in the induction of the lesions has not been investigated. Recently, Lapis (1978) observed liver tumours in 30-50% of chickens that had been inoculated perinatally with MC-29 RNA avian leukosis virus suspension. These liver tumours, which developed 25-45 days after inoculation with the virus, originated from paremchymal cells and showed malignant properties as evinced by invasion of adjacent tissues, penetration of blood vessels, metastasis to distant organs including lung and spleen, and transplantability to other avian hosts. Experience with the mouse leukemia virus has shown that the activation of the virus may not occur during the lifespan of the carrier host and the host may remain in good health, even though it carries the virus and transmits it to its progeny (Gross, 1954). A viral etiology of 'spontaneous' liver tumours could thus explain the observation that the incidence of liver tumours may alter from year to year in a given strain (Grasso & Hardy, 1974; Andervont, 1950).

The results of carcinogenicity tests have shown that phenobarbital treatment *following carcinogen administration* results mainly in an increased number of liver tumours (Peraino et al., 1971; Nishizumi, 1976). Similar results have been observed with other

microsomal enzyme inducers, such as DDT, BHT, PCBs and α-HCH (Peraino et al., 1971, 1973b, 1975, 1977; Nishizumi, 1976; Kimura et al., 1976; Schulte-Hermann, 1978). The underlying mechanism for the induction of liver tumours in susceptible animal species by microsomal enzyme inducers, therefore, seems to be a promoting effect on initiated cells. Kunz et al. (1978) observed an increase in pre-neoplastic areas in the liver *only* with doses of phenobarbital sufficient to cause liver enlargement and *only* during the initial phase of drug-mediated cell proliferation. These results strongly suggest that tumour-promoting effects by microsomal enzyme inducers are due to the capacity of these compounds to induce liver cell hyperplasia. The mechanism of action might thus be the same as can be observed when partial hepatectomy is used for promotion of tumour development (Kitigawa, 1971; Craddock, 1971; Scherer et al., 1972). The observation that microsomal enzyme inducers, such as diphenylhydantoin and amobarbital, which do not cause liver enlargement, show no tumour-promoting activity (Peraino et al., 1975) provides additional support for this hypothesis. Recent studies by Ohde et al. (1979) have shown that the administration of microsomal enzyme inducers, such as α-HCH or cyproterone acetate (CPA), to rats previously treated with DENA resulted in a dramatically increased rate of proliferation of pre-neoplastic cells, whilst cell division in the surrounding (normal) parenchyma was much less pronounced. It would seem therefore that pre-neoplastic cells are extremely susceptible to the effects of liver mitogens which may pose considerable difficulties in the assessment of safe levels of microsomal enzyme inducers in the human situation.

The results of the present investigations indicate that dieldrin administration to mice caused pronounced generalized liver enlargement, which was due to a combination of liver cell hyperplasia and hypertrophy. The occurrence of liver cell hyperplasia was evinced by increases in the total DNA content of the liver. Increases in total liver protein/liver DNA quotients, in liver microsomal protein/liver DNA quotients and in liver soluble protein/liver DNA quotients on the other hand, were indicative of liver cell hypertrophy and of proliferation of hepatocellular SER. However, hyperplasia was shown to play a more prominent role in the overall liver enlargement than hypertrophy.

In the case of non-dieldrin treated mice and using data obtained in mice maintained on semi-synthetic diet and filter paper bedding as the baseline, the administration of conventional diet and exposure to sawdust bedding caused only marginal enlargement of the liver. There was no evidence for the occurrence of liver cell hyperplasia and any liver enlargement was, consequently, due exclusively to hypertrophy. The rationalization that enhancement of the incidence of liver tumours in CF-1 mice may be caused by the induction of liver cell hyperplasia is, therefore, consistent with the observation that dieldrin administration to mice caused pronounced hyperplastic effects followed by the early occurrence of liver tumours, whereas conventional diet and sawdust bedding, which did not induce liver cell hyperplasia, showed no overt tumour-promoting activity.

The results obtained with dieldrin in various strains and species (Wright et al., 1972) have led to the suggestion that the induction of liver cell hyperplasia in mouse liver could be related to a limited capacity of mouse hepatocytes to respond effectively to an increased functional demand for oxidative microsomal enzyme systems. The induction of rat liver mono-oxygenase(s) and epoxide hydratase by dieldrin is more pronounced in rats than in mice. Vaino & Parkki (1976) observed a seven-fold increase in the specific

activity of liver *p*-nitroanisole *O*-demethylase and a five-fold induction of liver epoxide hydratase activity in rats exposed by the intraperitoneal route to 10 mg dieldrin.kg^{-1} bodyweight for only six days. Rapid and pronounced induction of liver mono-oxygenase activity in rats exposed to dieldrin was also observed by Wright et al. (1972). Metabolic data (Hutson, 1976) also indicate that the hydroxylation of dieldrin (which is a major metabolic pathway) is less efficient in mice than in rats and that the concentrations of dieldrin in liver and other tissues are consequently higher in mice.

In contrast to results obtained in CF-1 mice, it has been shown that total liver DNA did *not* increase in rats fed on 200 mg dieldrin.kg^{-1} diet for up to 28 days (Wright et al., 1972). This finding coupled with the observed increases in liver protein/liver DNA quotients in test animals, showed that the increases in relative liver weight in these rats were due to liver cell hypertrophy rather than to an increase in the number of hepatocytes. The increase in the size of hepatocytes was largely accounted for by increases in microsomal protein and soluble protein (up to 74% of control values). This was confirmed by electron microscopic observations that proliferation of SER of rat liver cells was pronounced shortly after exposure to dieldrin.

If the induction of liver cell hyperplasia by microsomal enzyme inducers is related to limitations in the capacity of the animal to respond effectively to an increased functional demand for drug-metabolizing enzyme systems in liver cells, one might assume that the most potent microsomal enzyme inducers should induce the greatest degree of liver cell proliferation. However, studies in rats with groups of chemically related compounds, e.g. derivatives of BHT (Gilbert et al., 1969) and HCH-isomers (Schulte-Hermann, 1974a) revealed that some of the compounds tested were strong inducers of drug-metabolizing enzymes, but weak inducers of liver cell hyperplasia, in this species. Furthermore, when the inducing potentials of chemically unrelated inducers such as phenobarbital, α-HCH and DDT were compared, liver enlargement in the rat was not invariably related to enzyme induction (Kunz et al., 1966a; Koransky et al., 1969). It appears, therefore, that liver cell hyperplasia is not a necessary consequence of pronounced induction of drug-metabolizing enzyme systems.

There is also evidence to indicate that the two effects of chemical inducers, i.e. liver enlargement and induction of drug-metabolizing enzyme systems, are not necessarily coupled. Aminotriazole, an inhibitor of heme biosynthesis, prevented the phenobarbital-induced increase of cytochrome P-450, but the increase in liver size and proliferation of SER were not hindered (Raisfeld et al., 1970). This observation suggests that liver enlargement can be uncoupled from multiplication of the heme moiety of the mixed-function oxidase. Recent work by Schulte-Hermann (1977) suggests that the inductive pathways leading to cell proliferation and mono-oxygenase multiplication diverge a few hours after initiation of the inductive process. This is evinced by the following experimental results:

1. The increase in liver DNA synthesis requires the consumption of dietary protein 5-8 hours before the initiation of DNA replication and can be blocked by a low dose of actinomycin D (50 μg.kg^{-1} bodyweight) in the first few hours after the administration of α-HCH.
2. In contrast, the increase of cytochrome P-450 and of *N*-demethylation do not require dietary protein and are not sensitive to even higher doses of actinomycin D (500 mg.kg^{-1}

bodyweight).

There are indications of species differences with respect to the induction of liver cell hyperplasia by microsomal enzyme inducers (Wright et al., 1972, 1978) but information on the susceptibility of the human liver to the effects of these compounds is rather limited. Results obtained with rhesus monkeys exposed to dieldrin (Wright et al., 1978) and the absence of any detectable changes in the livers of humans exposed to high endogenous concentrations of dieldrin point to a slow rate of metabolic clearance of dieldrin in these primate species and to a low sensitivity of their livers to this compound (Hunter & Robinson, 1967; Hunter et al., 1969; Jager, 1970). The dietary intake required for the induction of the rhesus monkey liver microsomal mono-oxygenase system was 25-30 $\mu g.kg^{-1}$ bodyweight.day^{-1}, which is approximately 300 times greater than that of the general human population in 1966-1967 (Wright et al., 1978). The corresponding threshold concentration in the liver was 6 to 7 μg dieldrin.g^{-1}; this concentration would be associated with marked microsomal enzyme induction and overt liver enlargement in livers of rats and mice. There was no evidence for the occurrence of liver hyperplasia in monkeys fed dieldrin at dietary concentrations from 0.01-5 $mg.kg^{-1}$ for up to 6.5 years (Wright et al., 1978).

In the case of dieldrin, therefore, it would seem that mouse liver is more susceptible to the mitogenic effects of this compound than primate liver. As a result it might be argued that the tumour-promoting effect of microsomal enzyme inducers is of secondary importance in the human situation, since chronic drug treatment sufficiently high to induce liver cell proliferation in humans might only be conceivable with a few substances. Over the past 5 years, however, there has been a surprising increase in the number of published reports of patients with liver adenomas, and a close relationship has been found with the consumption of oral contraceptives (Sherlock, 1978). In view of the very large number of women taking oral contraceptives and the small numbers of primary liver tumours that are being reported (Klatskin, 1977), the risk of this complication is not very great. However, it has been argued (Sherlock, 1978) that the proportion of women who have taken the hormone for more than 5 years is much smaller, and this is when the risk usually arises. Furthermore, the observation that pre-neoplastic liver cells are more susceptible to the mitogenic effects of microsomal enzyme inducers (Ohde et al., 1979) than normal hepatocytes indicates that humans with pre-neoplastic liver lesions might be at risk at low levels of exposure to such compounds.

In the present study, chronic exposure of male CF-1 mice to 10 mg dieldrin.kg^{-1} diet resulted in the appearance of liver tumours after approximately 43 weeks. On the basis of previous studies with dieldrin in the CF-1 mouse (Walker et al., 1973), it would seem that in utero exposure of mice to the compound reduced the latent period for the development of hepatocellular carcinomata (type B tumours). Nearly 50% of mice with these tumours killed or died between 18 months and the termination of the study after 26 months showed lung metastasis. Liver tumours also occurred in some mice in non-dieldrin treatment groups. However, these lesions were much smaller than those observed in the dieldrin treatment groups being usually less than 1 cm in diameter (compared with 2-5 cm diameter of most liver tumours in dieldrin treatments). Two liver tumours from the non-dieldrin groups showed morphological characteristics of hepatocellular carcinomata, but no lung metastases were observed in any of the non-dieldrin treated animals bearing liver tumours.

Comparative biochemical investigations in non-nodular and nodular liver tissue from dieldrin-treated mice revealed that mono-oxygenase, epoxide hydratase and glutathione *S*-epoxide transferase activities were slightly, but consistently higher in nodular liver tissues. Discontinuation of dieldrin administration to 85-week-old mice with liver tumours resulted in virtually complete regression of enzyme induction in non-nodular liver tissue. In contrast, regression of enzyme activity was less complete in nodular liver tissue, even though no dieldrin could be detected in the tissue.

The reversibility experiment also showed that hyperplasia persisted in non-nodular liver tissue throughout the regression. Persistence of liver cell hyperplasia during regression was also reported in studies with α-HCH and BHT (Schulte-Hermann et al., 1971). Thus, in contrast to the effects on drug-metabolizing enzymes, the induction of liver cell hyperplasia by microsomal enzyme inducers could represent an irreversible process.

Liver tumours were found to be deficient in G-6-Pase activity and also showed low concentrations of glutathione. G-6-Pase deficiency is a common feature of pre-neoplastic liver lesions induced by chemical carcinogens, such as DENA (Scherer et al., 1972; Scherer & Emmelot, 1975, 1976). In the current study, a slight depression of the specific activity of liver G-6-Pase, i.e. the activity expressed per unit weight of microsomal protein, was also observed in livers of dieldrin-treated mice *before* the occurrence of liver tumours (after 15 weeks of exposure to dieldrin). However, when the enzyme activity was expressed per unit liver DNA, which reflects the enzyme concentration per liver cell, no consistent effect was observed. Depression of liver G-6-Pase has been observed after treatment with a number of hepatotoxic compounds (Feuer et al., 1965). However, the results obtained in this study suggest that dieldrin-exposure does not cause any depression of hepatocellular G-6-Pase in male CF-1 mice and that the observed trend towards decrease in the specific activity of this enzyme, i.e. activity expressed in terms of unit weight of microsomal protein, probably reflects the occurrence of liver cell hypertrophy in dieldrin-treated mice. This hypertrophy may also explain the depression of centrilobular G-6-Pase observed in rats exposed to other microsomal enzyme inducers, e.g. phenobarbital and BHT (Crampton et al., 1977).

The depression of glutathione in liver tumours observed in the present study could also be a general characteristic of neoplastic tissue. Fiala and his associates (1973, 1976) have demonstrated that the induction of pre-neoplastic liver lesions is associated with the activation of γ-glutamyl transpeptidase (γ-GT) in these cells. Orlowski & Meister (1970) have postulated that γ-GT and glutathione are involved in the transport of amino acids across the cellular membrane. It has been suggested (Fiala et al., 1976), that pre-neoplastic hepatocytes are unable to supply enough ATP to maintain both a high concentration of glutathione and a high γ-GT activity, which requires a high turnover of glutathione. As a result, their glutathione concentrations may never approach those of normal hepatocytes.

In conclusion, microsomal enzyme inducers, such as dieldrin, DDT, phenobarbitone and various stereoisomers of HCH, may cause liver tumours in experimental animals by facilitating the expression of pre-existing oncogenic factors. The most likely mechanism of action is that these compounds are capable of inducing liver cell hyperplasia through which the proliferation of pre-existent pre-neoplastic liver lesions may be profoundly

increased.

This rationalization is consistent with the observation that conventional diet and sawdust bedding, which were shown to contain agents that induce the liver microsomal mono-oxygenase system but failed to induce liver hyperplasia in the CF-1 mouse, had no overt tumour-promoting activity.

Summary

A variety of xenobiotic compounds are known to induce characteristic changes in the livers of laboratory animals. These changes include liver enlargement, usually as a result of cell enlargement (hypertrophy) or cell replication (hyperplasia), induction of drug metabolizing enzymes and proliferation of the smooth endoplasmic reticulum (SER). Such changes are not usually accompanied by evidence of liver damage and in such cases are reversible upon withdrawal and elimination of the compound. Consequently, most authors regard this phenomenon as an adaptive response of the organ to increased functional demands. However, chronic exposure of various strains of mice to dieldrin, phenobarbitone, DDT and the α-, β- and γ- stereoisomers of hexachlorocyclohexane (HCH, also known as benzenehexachloride, BHC) may lead to the development of liver tumours.

There is no apparent relationship in chemical structure between these compounds and their main common feature is that they are inducers of the microsomal mono-oxygenase system of mammalian liver. This has led to the suggestion that a common property of microsomal enzyme inducers may be to enhance the incidence of liver tumours in susceptible animal species, possibly as a result of an increased rate of metabolic activation of environmental pre-carcinogens.

The objective of this study was to ascertain whether microsomal enzyme inducers were present in commercial rodent diet and softwood bedding employed routinely in this laboratory and to establish what effects such naturally occurring agents may have on the incidence of liver tumours in dieldrin-treated (10 $mg.kg^{-1}$ diet) and non-dieldrin treated CF-1 mice using animals maintained on semi-synthetic diet and filter paper bedding as controls.

CF-1 mice were bred and reared on the experimental treatments to ensure continuous exposure during both the pre-natal and post-natal periods.

The administration of dieldrin to CF-1 mice resulted in pronounced generalized liver enlargement due to a combination of hypertrophy and hyperplasia. Hyperplasia was a major contributory factor in the observed liver enlargement. In many dieldrin-treated mice the increases in liver DNA were nearly proportional to the increases in liver weight.

Liver enlargement induced by dieldrin was accompanied by the induction of drug-metabolizing enzymes, e.g. *p*-nitroanisole *O*-demethylase (used as an index of mono-oxygenase activity), epoxide hydratase, glutathione *S*-epoxide transferase and UDP-glucuronyl transferase activity.

In the case of non-dieldrin treated mice and using data obtained in mice maintained on semi-synthetic diet and filter paper bedding as the baseline, the administration of conventional diet to mice and exposure to sawdust bedding, both singly and in combination, caused only marginal enlargement of the liver. There was no evidence for hyperplasia and any liver enlargement would thus appear to be due exclusively to hypertrophy. These environmental factors did cause induction of the liver mono-oxygenase system although

this effect was less pronounced than that produced by dieldrin. Other drug-metabolizing enzymes were only very slightly increased by conventional diet and sawdust bedding.

Dieldrin administration to mice resulted in the relatively early appearance of nodular hepatic tumours, the first being observed in a mouse aged approximately 43 weeks. At 65 weeks, the incidence of liver tumours in the dieldrin treatment groups was very high, even in animals maintained on a semi-synthetic diet and filter paper bedding. Longer-term exposure to dieldrin resulted in the development of hepatocellular carcinomata. Forty-eight percent of mice with hepatocellular carcinomata ('B' type tumours) that died or were killed between 18 months and the termination of the study showed lung metastases.

On the basis of previous studies it would seem that in utero exposure of CF-1 mice to dieldrin reduced the latent period for the development of liver tumours. The fact that the incidence of liver tumours in dieldrin-treated animals maintained on semi-synthetic diet and filter paper bedding was similar to that observed in other dieldrin treatment groups indicates that it is unlikely that dieldrin exerts its action on mouse liver by facilitating or exacerbating the action of a potent environmental carcinogen.

Liver tumours also occurred in some mice from the groups not treated with dieldrin. However, these tumours were much smaller than those observed in the dieldrin groups being usually less than 10 mm in diameter. Two tumours from the non-dieldrin groups showed morphological characteristics of hepatocellular carcinomata. No lung metastases were observed in non-dieldrin treated animals bearing liver tumours.

Conventional diet and sawdust bedding did not exert any obvious influence on the development of 'spontaneous' tumours in the livers of male CF-1 mice and it would seem that, even though conventional diet and sawdust contained agents that induced the mouse liver microsomal mono-oxygenase system, these agents have no intrinsic tumour-promoting activity or are present at concentrations below the threshold for overt tumour-promoting action.

The fact that nodular lesions were observed in a few animals maintained on semi-synthetic diet and filter paper bedding confirms the presence of a pre-existing oncogenic factor or susceptibility in this strain of mouse. This observation, together with evidence that dieldrin and its mammalian metabolites possess neither mutagenic activity or potential is consistent with the concept that dieldrin acts by facilitating the expression of this pre-existing factor, probably by inducing hyperplasia in mouse liver. This rationalization is consistent with the more general concept that tumour-promoting agents act by inducing growth (hyperplasia) in their target organs.

Comparative investigations in non-nodular and nodular liver tissue from the same animals revealed that mono-oxygenase, epoxide hydratase and glutathione *S*-epoxide transferase activities were slightly, but consistently higher in nodular liver tissue. A marked depression of G-6-Pase activity and glutathione concentration was observed in nodular liver tissue.

The withdrawal of dieldrin from the diet and subsequent metabolic elimination of the compound resulted in complete regression of the activities of drug-metabolizing enzymes in non-nodular liver tissue. Regression of enzyme activity also occurred in nodular liver tissue but control rates were not attained even though no dieldrin could be detected in the tissues.

No changes were observed in the total DNA content of non-nodular liver tissue during regression which suggests that the induction of liver cell hyperplasia by microsomal enzyme inducers may be irreversible.

Samenvatting

Van een groot aantal uiteenlopende lichaamsvreemde stoffen (xenobiotica) is bekend dat zij in bepaalde doseringen karakteristieke veranderingen teweeg kunnen brengen in levers van proefdieren (zie hoofdstuk 1). Deze veranderingen bestaan gewoonlijk uit leververgroting als gevolg van een combinatie van celvergroting (hypertrofie) en celvermeerdering (hyperplasie), inductie van enzymen die een rol spelen bij het metabolisme van lichaamsvreemde stoffen (biotransformatie-enzymen; Engels: drug-metabolizing enzymes) alsmede proliferatie van het gladde (Engels: smooth) endoplasmatische reticulum (SER). Aanwijzingen voor leverbeschadiging zijn in de meeste gevallen niet waargenomen en de resultaten wijzen er tevens op dat de veranderingen zeer waarschijnlijk reversibel van aard zijn wanneer de blootstelling van het proefdier aan de lichaamsvreemde stof wordt gestopt. Dientengevolge worden deze veranderingen door velen beschouwd als een aanpassing (adaptatie) van de lever aan een verhoogde funktionele belasting. Recente toxiciteitsproeven met muizen hebben echter uitgewezen dat blootstelling aan een aantal van deze lichaamsvreemde stoffen, zoals DDT, dieldrin, phenobarbital en verschillende stereoisomeren van hexachlorocyclohexaan (HCH), aanleiding kunnen geven tot de ontwikkeling van levertumoren.

Er bestaat geen duidelijke samenhang in chemische structuur tussen deze tumorigene lichaamsvreemde stoffen. Deze stoffen hebben echter wel met elkaar gemeen dat zij de activiteit van microsomale biotransformatie-enzymen en in het bijzonder de activiteit van het mono-oxygenasesysteem in de lever verhogen (inductie). Dit heeft geleid tot de hypothese dat de inducerende eigenschappen van deze stoffen van betekenis zouden zijn bij het ontstaan van levertumoren in proefdieren. Deze hypothese ontleent steun aan het feit dat biotransformatie-enzymen een belangrijke rol spelen bij de cellulaire activatie van carcinogene reactiviteit uit pre-carcinogenen.

Het doel van de in dit proefschrift beschreven studie was te onderzoeken welke invloed omgevingsgebonden factoren, n.l. het commerciële proefdierdieet en zaagselbedding, kunnen uitoefenen op het vóórkomen van levertumoren in CF-1-muizen. Uit de literatuur is namelijk bekend dat deze exogene factoren *natuurlijke voorkomende* microsomale enzym inductoren bevatten. Deze stoffen zouden in analogie met *lichaamsvreemde* microsomale enzym inductoren een verhogend effect kunnen uitoefenen op het vóórkomen van levertumoren in CF-1-muizen. De effecten van een conventioneel dieet (CD) en van zaagselbedding (S) werden in dit onderzoek bestudeerd aan de hand van een controlegroep CF-1-muizen die werden gevoed op een semi-synthetisch dieet (SSD) en gehuisvest in kooien met verknipt filtreerpapier als bedding (F). Als positieve controle werd in sommige behandelingsgroepen aan het voedsel 10 mg.kg^{-1} van het pesticide dieldrin, een enzym-inducerende stof die tevens het vóórkomen van levertumoren in CF-1-muizen verhoogt, toegevoegd. CF-1-muizen (mannelijk geslacht) werden gefokt conform de experimentele behandelingswijzes, zodat blootstel-

ling plaats vond gedurende de *pre-* en *post*-natale periodes. Hiertoe werden de moederdieren 14 dagen lang op de verschillende behandelingswijzes geplaatst voordat de conceptie plaats kon vinden. Het nageslacht verbleef bij de moederdieren tot de speentijd (ca. 3 weken na de geboorte); op dit tijdstip werden de moederdieren en het vrouwelijk nageslacht gedood, waarna het mannelijk nageslacht werd gehandhaafd op de gevolgde experimentele behandelingswijzes.

De resultaten van het biochemisch onderzoek (zie hoofdstuk 3) toonden aan dat chronische blootstelling van CF-1-muizen aan 10 mg dieldrin.kg^{-1} een leververgroting van 40-60% veroorzaakte, die gepaard ging met het voorkomen van zowel hypertrofie (= celvergroting) en hyperplasie (= celvermeerdering). Het laatste fenomeen bleek de belangrijkste bijdrage te leveren tot de waargenomen leververgroting. Dit werd afgeleid uit de verhoging van het totale DNA-gehalte van de lever, dat in een groot aantal met dieldrin behandelde dieren proportioneel steeg met het levergewicht. In met dieldrin behandelde dieren ging de vergroting van de lever vergezeld van activiteitsverhogingen van biotransformatie-enzymen. De activiteit van *p*-nitroanisol *O*-demethylase, dat werd gemeten als een graadmeter van de mono-oxygenaseactiviteit in de lever, was na 15 weken behandeling 3 tot 4 keer zo hoog in met dieldrin behandelde muizen. Tevens werden 2-voudige verhogingen van de activiteit van glutathion *S*-epoxide transferase en epoxide hydratase waargenomen, terwijl de activiteit van UDP-glucuronyl transferase met een factor 1.5 was gestegen. Inductie van biotransformatie-enzymen in met dieldrin behandelde dieren werd ook waargenomen na langere blootstellingsduur (na 52 en 68-72 weken) en het patroon van de activiteitenverhogingen verschilde niet wezenlijk van dat na 15 weken behandeling.

Uit het onderzoek is gebleken dat CD en S slechts een geringe leververgroting veroorzaakten (<10%). De DNA-metingen leverden geen aanwijzigingen op voor het optreden van hyperplasie, zodat de door deze factoren veroorzaakte leververgroting aan hypertrofische veranderingen van levercellen moet worden toegeschreven. CD en S veroorzaakten echter wel een ca. 2-voudige verhoging van de activiteit van *p*-nitroanisol *O*-demethylase in de lever, hetgeen de aanwezigheid van microsomale enzym inductoren in dieet en bedding bevestigt. De door CD en S veroorzaakte stijging van de activiteit van *p*-nitroanisol *O*-demethylase was echter aanmerkelijk minder sterk dan in muizen die met dieldrin werden behandeld. Bovendien bleek dat de door deze factoren veroorzaakte enzym inductie niet additief was. De activiteit van andere biotransformatie-enzymen, zoals epoxide hydratase, glutathion *S*-epoxide transferase en UDP-glucuronyl transferase, werden niet of in zeer geringe mate gestimuleerd door CD en/of S.

Continue blootstelling van CF-1-muizen aan 10 mg.dieldrin kg^{-1} voedsel resulteerde reeds na ca. 43 weken in het ontstaan van tumoren in de lever. Het vóórkomen van levertumoren in de met dieldrin behandelde CF-1-muizen varieerde na 65 weken van 73.3% in muizen op SSD en F tot 100% in muizen op SSD en S. In het verloop van de proef bleek dat de meeste nodulaire lesies in met dieldrin behandelde dieren zich ontwikkelden tot hepatocellulaire carcinomata. Het maligne karakter kon onder andere worden afgeleid uit het feit dat bijna 50% van de muizen met hepatocellulaire carcinomata, die stierven of werden gedood tussen 78 weken en het einde van de proef na 110 weken, metastasen hadden in de long. Het vroegtijdig ontstaan van levertumoren zou verband kunnen houden met het feit dat in de hier beschreven studie CF-1-muizen reeds in utero werden blootgesteld aan dieldrin.

Het feit dat het hoge vóórkomen van levertumoren ook werd waargenomen in muizen die op SSD en F werden gehandhaafd, duidt erop dat het niet waarschijnlijk is dat de tumorigene effecten van dieldrin berusten op een versterking van de werking van omgevingsgebonden carcinogenen, zoals aflatoxines en/of nitrosamines.

Levertumoren kwamen eveneens voor in *niet* met dieldrin behandelde CF-1-muizen. Deze tumoren ontstonden op een later tijdstip dan in met dieldrin behandelde muizen en waren tevens geringer in omvang (meestal <0.5 cm tegenover een omvang van 2-5 cm in met dieldrin behandelde CF-1-muizen). Slechts in enkele gevallen bestonden er morfologische aanwijzingen voor een maligne karakter. Metastasen in de long werden niet waargenomen. Een duidelijke invloed van CD en/of S op het vóórkomen van levertumoren in CF-1-muizen kon niet worden vastgesteld. Tegen het einde van de proef ontstonden meer levertumoren in muizen op SSD en S, dan in muizen die op SSD en F werden gehandhaafd. Een promoverend effect van S op het ontstaan van levertumoren in de CF-1-muis kon hieruit niet worden afgeleid aangezien geen verschillen werden waargenomen tussen het vóórkomen van levertumoren in de behandelingsgroepen CD+F en CD+S.

Uit deze resultaten blijkt dat ondanks het feit dat CD en S een verhoging van de activiteit van het mono-oxygenasesyteem in levers van CF-1-muizen veroorzaken, er van deze factoren *geen* promoverend effect op het ontstaan van levertumoren uitgaat. In het huidige onderzoek werden levertumoren ook waargenomen in CF-1-muizen op SSD en F. Alhoewel ook onder deze experimentele omstandigheden niet kan worden uitgesloten dat blootstelling aan exogene carcinogene stoffen plaats vond, lijkt het niet waarschijnlijk dat dergelijke tumoren worden veroorzaakt door exogene factoren. Op grond van de uitkomsten van dit onderzoek wordt verondersteld dat het ontstaan van 'spontane' levertumoren het gevolg is van de expressie van endogeen oncogeen potentiaal dat genetisch is vastgelegd en mogelijkerwijs in oorsprong wordt veroorzaakt door een viraal genoom. De promoverende invloed van microsomale enzyminductoren op het ontstaan van levertumoren zou derhalve kunnen berusten op de versterking van de expressie van pre-existente endogene oncogene factoren. Voor het meest waarschijnlijke werkingsmechanisme, nl. versnelling van de groei van pre-neoplastische levercellen als gevolg van de hyperplasiogene (mitogene) effecten van microsomale enzyminductoren, worden verschillende ondersteunende argumenten genoemd in dit proefschrift, waarvan het belangrijkste wellicht is, dat de mitogene effecten van microsomale enzyminductoren in veel sterkere mate tot uitdrukking komen in pre-neoplastische levercellen dan in het normale parenchym.

Vergelijkend onderzoek in non-nodulair en nodulair leverweefsel van met dieldrin behandelde dieren toonde aan dat de activiteit van verschillende biotransformatie-enzymen iets hoger was in nodulair leverweefsel. Een sterke daling werd echter in dit weefsel waargenomen in de activiteit van glucose-6-phosphatase en in de concentratie van glutathion. Het stopzetten van de blootstelling van 85 weken oude CF-1-muizen aan dieldrin resulteerde in een volledige afname van de activiteit van biotransformatie-enzymen in non-nodulair leverweefsel tot het niveau dat in controledieren werd waargenomen. Een minder volledige regressie van enzymactiviteit vond plaats in nodulair leverweefsel. Er bleek geen samenhang te bestaan met het in de verschillende weefsels nog aanwezige dieldrinresidu, aangezien in zowel het non-nodulaire als ook in het nodulaire leverweefsel geen dieldrin meer kon worden aangetoond. De door dieldrin geïnduceerde leverhyperplasie bleef voortbestaan

in non-nodulair leverweefsel nadat de blootstelling aan dieldrin was stopgezet, hetgeen erop wijst dat de inductie van leverhyperplasie door lichaamsvreemde stoffen naar alle waarschijnlijkheid niet reversibel is.

Table 1. Threshold doses for the induction of liver enlargement. Source: Schulte-Hermann (1974a).

Compound	Threshold dose	Application	Animal species	References
phenobarbital	30 mg.kg^{-1} bodyweight	daily in drinking water	mouse	Kunz et al., 1966a
pyrethrum	85 mg.kg^{-1} bodyweight	daily	rat	Springfield et al., 1973
α-HCH	25 mg.kg^{-1} bodyweight	once	rat	Schulte-Hermann et al., 1974b
	10 mg.kg^{-1} diet	daily	rat	Fitzhugh et al., 1950
DDT	128 mg.kg^{-1} bodyweight	daily	rat	Hoffman et al., 1970
dieldrin	0.05–1.0 mg.kg^{-1} diet	daily	rat	Fitzhugh et al., 1964 Walker et al., 1969
	0.05 mg.kg^{-1} bodyweight	daily	dog	Walker et al., 1969
BHT	75 mg.kg^{-1} bodyweight	daily	rat	Gilbert & Golberg, 1965

Table 2. Incidence of liver tumours in CF-1 mice fed dieldrin for 132 weeks. Reference: Walker et al. (1973).

Dietary concentration (mg.kg^{-1})	Number of animals	% with liver tumours		
		Type A[1]	Type B[1,2]	Total A + B
males				
0	288	16	4	20
0.1	124	22	4	26
1.0	111	23	8	31
10.0	176	37	57	94
females				
0	297	13	0	13
0.1	90	23	4	27
1.0	87	31	6	37
10.0	148	37	55	92

1. Liver tumours were classified as Type A (in which parenchymal structure is basically retained) and Type B (in which parenchymal structure is distorted).
2. Mice with Type B tumours frequently showed Type A tumours as well, but these have not been included in Column A.

Table 3. Incidence of liver tumours in male CF-1 mice fed 10 mg dieldrin.kg^{-1} diet for up to 64 weeks and surviving for 104 weeks. Reference: Walker et al. (1973).

Duration of feeding (weeks)	Number of animals	Number with liver tumours		
		Type A[1]	Type B[1]	Total A + B
0	18	2	0	2 (11%)
2	13	2	0	2 (15%)
4	10	0	1	1 (10%)
8	10	3	1	4 (40%)
16	11	4	0	4 (36%)
32	10	4	0	4 (40%)
64	13	6	7	13 (100%)

1. As in Table 2.

Table 4. Incidence of liver tumours in 3 strains of mice exposed to dieldrin for up to 2 years. Reference: Thorpe & Hunt (1975).

Strain	Sex	Dietary concentration (mg.kg^{-1})	Number of animals	Number with liver tumours			% With liver tumours
				Type A[1]	Type B[1]	Total A + B	
CF1	male	0	45	2	1	3	6
	male	10	30	4	12	16	53
CF1xLACG	male	0	45	2	1	3	6
	male	10	30	5	11	16	53
LACG	male	0	45	3	1	4	9
	male	10	29	4	3	7	24
CF1	female	0	44	11	2	13	29
	female	10	29	2	19	21	72
CF1xLACG	female	0	43	0	0	0	0
	female	10	30	2	10	12	40
LACG	female	0	45	0	1	1	2
	female	10	31	2	4	6	19

1. As in Table 2.

Table 5. Liver tumour incidence in two generations of CF-1 mice ($P + F_1$) exposed to DDT. Reference: Tomatis et al. (1972).

Treatment	Number of mice ($P + F_1$)	Incidence of liver tumours[1]	
		Number	%
males			
control	113	25	22
DDT 2 $mg.kg^{-1}$ diet	124	57	46
DDT 10 $mg.kg^{-1}$ diet	104	52	50
DDT 50 $mg.kg^{-1}$ diet	127	67	53
DDT 250 $mg.kg^{-1}$ diet	103	82	80
females			
control	111	4	4
DDT 2 $mg.kg^{-1}$ diet	111	4	4
DDT 10 $mg.kg^{-1}$ diet	124	11	9
DDT 50 $mg.kg^{-1}$ diet	104	13	13
DDT 250 $mg.kg^{-1}$ diet	90	69	77

1. The liver tumour incidence reported in this study is based on the number of mice surviving at the time of appearance of the first tumour at any site in each group (effective number of mice).

Table 6. Carcinogenicity studies with phenobarbitone in mice.

Strain	Sex	Duration	Dose	Number of mice	Liver tumour incidence		Reference
					Number	%	
CF-1	male	109	0 $mg.kg^{-1}$ diet	45	11	24	Thorpe & Walker (1973)
			500 $mg.kg^{-1}$ diet	30	24	80	
	female	109	0 $mg.kg^{-1}$ diet	44	10	23	
			500 $mg.kg^{-1}$ diet	28	21	75	
C3H	male	52	0 $mg.kg^{-1}$ diet	37	25	68	Peraino et al. (1973a)
			500 $mg.kg^{-1}$ diet	36	35	97	
	female	52	0 $mg.kg^{-1}$ diet	39	5	13	
			500 $mg.kg^{-1}$ diet	29	29	100	
CF-1	male	120	0	44	12	27[1]	Ponomarkov et al. (1976)
			0.05% in drinking water	98	77	78[1]	
	female	120	0	47	0	-[1]	
			0.05% in drinking water	73	45	62	

1. The liver tumour incidence in this study was based on the number of survivors at the time that the first tumour was observed.

Table 7. Carcinogenicity studies with hexachlorocyclohexane (HCH) in mice.

Strain	Sex	Dose ($mg.kg^{-1}$ diet)	Duration (weeks)	Number of mice	Incidence of liver tumours		Reference
					Number	%	
dd	male	0	24	14	0	-	Nagasaki et al.
		6.6 HCH[1]	24	20	0	-	(1971, 1972)
		66.0 HCH[1]	24	20	0	-	
		666.0 HCH[1]	24	20	20	100	
dd	male	0	24	20	0	-	Ito et al.
		100 α-HCH	24	20	0	-	(1973)
		250 α-HCH	24	38	30	79	
		500 α-HCH	24	20	20	100	
		100 β-HCH	24	20	0	-	
		250 β-HCH	24	20	0	-	
		500 β-HCH	24	20	0	-	
		100 γ-HCH	24	20	0	-	
		250 γ-HCH	24	20	0	-	
		500 γ-HCH	24	20	0	-	
		100 δ-HCH	24	20	0	-	
		250 δ-HCH	24	20	0	-	
		500 δ-HCH	24	20	0	-	
CF-1	male	0	110	45	11	24	Thorpe & Walker
		200 β-HCH	110	30	22	73	(1973)
		400 γ-HCH	110	29	27	93	
	female	0	110	44	10	23	
		200 β-HCH	110	30	13	43	
		400 γ-HCH	110	29	20	69	

1. Technical HCH, the composition of which was: 66.6% α-isomer, 11.3% β-isomer, 15.2% γ-isomer, 6.3% δ-isomer and 0.6% others.

Table 8. The incidence of liver nodules in male and female Wistar rats exposed to DDT or phenobarbitone-sodium (Ph-Na) for up to 152 weeks. Reference: Rossi et al. (1977).

Treatment	Initial number of animals[1]	Total number of animals with liver nodules	
		number	%
males			
DDT 500 $mg.kg^{-1}$ (2	26	9	34.6
Ph-Na 500 $mg.l^{-1}$ (3	22	13	59.0
females			
DDT 500 $mg.kg^{-1}$ (2	27	15	55.5
Ph-Na 500 $mg.l^{-1}$ (3	28	9	32.1

1. Survivors at time first liver nodule was observed in each group.
2. In the diet.
3. In the drinking water.

Table 9. Carcinogenicity studies with dieldrin in the rat.

Strain	Sex	Duration (weeks)	Dietary concentration ($mg.kg^{-1}$)	Number of animals[1]	Total animals with tumours		Remarks on liver lesions	Reference
					number	%		
Osborne Mendel	males and females[2]	104	0	17	3	18	enlarged centrilobular cells in dieldrin-treated rats, no liver cell tumours	Fitzhugh et al. (1964)
			0.5	22	8	36		
			2	23	8	35		
			10	18	4	22		
			50	20	4	20		
			100	18	3	17		
			150	11	0	-		
CFE	males	104	0	43	12	28	three females on 10 mg dieldrin. kg^{-1} and one control female showed focal hyperplasia forming microscopic hyperplastic nodules	Walker et al. (1969)
			0.1	23	6	26		
			1.0	23	5	22		
			10.0	23	8	35		
	females	104	0	43	19	44		
			0.1	23	15	65		
			1.0	23	14	61		
			10.0	23	12	52		
Osborne Mendel	males	110-111	0	10	5	50	low incidence of neoplastic lesions with no apparent increased frequency for treated groups over controls	NCI (1978a)
			29	46	24	52		
			65	50	22	44		
	females	110-111	0	10	7	70		
			29	47	39	83		
			65	48	27	56		
Fisher 344	males	104-105	0	24	24	100	two control males and four males on 50 mg dieldrin . kg^{-1} showed nodular hyperplasia which was classified as a non-neoplastic lesion	NCI (1978b)
			2	24	23	96		
			10	24	24	100		
			50	24	23	96		
	females	104-105	0	24	17	71		
			2	24	17	71		
			10	24	16	67		
			50	24	14	58		

1. Examined histologically.
2. Equally divided by sex.

Table 10. Liver tumour incidence in male Wistar rats treated with HCH. Reference: Ito et al. (1975).

HCH-isomer	Dietary concentration ($mg.kg^{-1}$)	Duration (weeks)	Liver tumours	
			Nodular hyperplasia	Hepatocellular carcinomata
α	1 500	72	10/13 (77%)	3/13 (23%)
α	1 000	72	12/16 (75%)	1/16 (6%)
α	1 000	48	5/12 (42%)	0/12 -
α	1 000	24	0/8 -	0/8 -
α	500	48	0/5 -	0/5 -
α	500	24	0/6 -	0/6 -
β	1 000	24	0/6 -	0/6 -
β	500	48	0/5 -	0/5 -
β	500	24	0/8 -	0/8 -
γ	500	48	0/8 -	0/8 -
γ	500	24	0/6 -	0/6 -
control		72	0/8 -	0/8 -

Table 11. Extent of binding of dieldrin or its metabolites to liver DNA. Reference: Wright et al. (1977).

Species (strain)	desintegrations. $min^{-1}.mg^{-1}$ DNA	Dieldrin pg equiv.mg^{-1} DNA	molecule equiv. 10^9 nucleotide $units^{-1}$
Rat (CFE)	0.614	1.92	1.52
Mouse (CF-1)	0.232	0.724	0.58
Mouse (LACG)	0.053	0.163	0.13

Individual animals in each group of 10 rats or mice received a single i.p. injection of $|^{14}C|$ -dieldrin in dimethylsulphoxide (0.1 ml) equivalent to 14.5 μCi (370 $\mu g.kg^{-1}$ bodyweight) for the rats and 1.45 μCi (400 $\mu g.kg^{-1}$ bodyweight) for the two mouse strains. The animals were killed by decapitation exactly 3 hours later. The livers were pooled according to species and strain.

Table 12. Experimental treatments. SSD = semi-synthetic diet; CD = conventional diet; F = filter paper; S = sawdust.

	Diet	Bedding	Dieldrin ($mg.kg^{-1}$ diet)
1	SSD	F	0
2	SSD	S	0
3	SSD	F	10
4	SSD	S	10
5	CD	F	0
6	CD	S	0
7	CD	F	10
8	CD	S	10

Table 13. Treatment groups sizes after weaning.

	Diet	Bedding	Dieldrin ($mg.kg^{-1}$ diet)	Treatment size
1	SSD	F	0	85
2	SSD	S	0	60
3	SSD	F	10	79
4	SSD	S	10	47
5	CD	F	0	93
6	CD	S	0	113
7	CD	F	10	100
8	CD	S	10	64
Total				614

Abbreviations as in Table 12.

Table 14. Composition per 100 g of Laboratory Animal Diet no. 2. Based on information supplied by the manufacturer.

Chemical composition		
moisture	8	g
ether extract	4.5	g
crude protein	21.5	g
crude fibre	2.7	g
total digestible nutrients	78	g
Vitamin and mineral composition		
lysine	1.1	g
methionine	0.39	g
calcium	0.9	g
phosphorus	0.8	g
vitamin A	1100	i.u.
vitamin D_3	120	i.u.
α-tocopherol (E)	2.4	i.u.
vitamin K_3	1.0	mg
riboflavin (B_2)	0.7	mg
pyridoxine (added) (B_6)	0.1	mg
pantothenic acid	1.7	mg
nicotinic acid (niacin)	8.0	mg
folic acid	0.02	mg
choline chloride (added)	45.0	mg
cyanocobalamin (B_{12})	1.5	µg
manganese	6.5	mg
iron	10.0	mg
iodine	0.05	mg
copper	2.0	mg
zinc	4.0	mg
cobalt	0.1	mg

digestible energy: 14.3 $kJ.g^{-1}$

Table 15. Composition of semi-synthetic diet (per 100 g).

protein	casein	23.6	g
carbohydrates	corn starch	46.7	g
	potato starch	10.0	g
	sucrose	5.0	g
fat	corn oil	10.0	g
vitamins	thiamine (B_1)	0.4	mg
	riboflavine (B_2)	0.5	mg
	pyridoxine-HCl (B_6)	0.6	mg
	nicotinic acid (niacin)	1.0	mg
	calcium pantothenate	1.2	mg
	biotin (H)	0.1	mg
	menapthone (K_3)	0.2	mg
	α-tocopherol (E)	7	i.u.
	vitamin D_3	100	i.u.
	vitamin A	500	i.u.
	cyanocobalamin (B_{12})	2	µg
	choline chloride	100	mg
minerals	calcium citrate	47.6	mg
	potassium dihydrogen phosphate	1.36	g
	magnesium carbonate	141	mg
	magnesium sulphate.7 H_2O	338	mg
	manganous sulphate.4 H_2O	18.4	mg
	zinc carbonate	4.0	mg
	ammonium ferric citrate	45.0	mg
	copper sulphate	2.6	mg
	sodium fluoride	0.5	mg
	potassium iodate	0.3	mg
	calcium hydrogen orthophosphate	1.05	g
	calcium carbonate	910	mg
	sodium chloride	700	mg

Metabolizable energy (calculated): 18.3 $kJ.g^{-1}$.

Table 16. Bodyweight[1],[2]. The results are expressed in g.

Treatments			Exposure time (weeks)							
diet	bedding	dieldrin (mg.kg^{-1} diet)	n[3]	15	n	52	n	68-72	n	92
SSD	F	0	4	34.2±0.8[4]	4	39.9±4.5	3	38.2± 1.7	6	42.8±7.2
		10	4	35.4±1.9	6	41.4±3.0	8	38.5± 4.5		
SSD	S	0	4	35.1±0.6	5	40.6±7.5	3	42.7± 4.1	6	37.4±5.2
		10	4	33.8±1.9	6	41.6±5.0	5	46.8± 5.1*		
CD	F	0	4	39.1±1.7**	4	42.2±6.5	2	45.4;39.0	6	44.7±2.9
		10	4	36.4±1.8	6	46.6±5.0*	6	42.8± 5.1		
CD	S	0	4	35.8±4.1	5	44.9±4.1	2	46.8;50.3**	6	40.3±5.8
		10	4	37.8±1.7**	6	46.8±4.2*	4	41.2± 1.8		

Abbreviations as in Table 12.
1. Significance of the difference between treatment and control mean (= SSD + F + 0 mg dieldrin.kg^{-1}): *p < 0.05; **p < 0.01; ***p < 0.001.

2. Dieldrin versus non-dieldrin statistical analysis:

	Exposure time (weeks)		
	15	52	68-72
SSD + F : 0 mg.kg^{-1} v 10 mg.kg^{-1}	n.s.	n.s.	n.s.
SSD + S : 0 mg.kg^{-1} v 10 mg.kg^{-1}	n.s.	n.s.	n.s.
CD + F : 0 mg.kg^{-1} v 10 mg.kg^{-1}	n.s.	n.s.	n.s.
CD + S : 0 mg.kg^{-1} v 10 mg.kg^{-1}	n.s.	n.s.	n.s.

*p < 0.05; **p < 0.01; ***p < 0.001; n.s.: not significant (p > 0.05).

3. Number of individual observations.
4. Mean ± standard deviation.

Table 17. Relative liver weight[1,2]. The results are expressed in g liver.100 g^{-1} bodyweight.

Treatments			Exposure time (weeks)									
diet	bedding	dieldrin ($mg.kg^{-1}$ diet)	15		52				68-72			92	
			n[3]	non-nodular livers	n	non-nodular livers	n	nodular livers	n	non-nodular livers	n	nodular livers	n	non-nodular livers
SSD	F	0	4	4.2±0.2[4]	4	4.1±0.4			3	4.8± 0.5			6	4.2±0.3
		10	4	6.4±0.2***	6	6.4±1.3**			5	9.0± 1.6**	3	12.2±0.3		
SSD	S	0	4	4.6±0.1*	5	4.2±0.3			3	4.1± 0.2			6	5.0±0.5**
		10	4	7.2±0.4***	4	7.8±1.9**	2	18.1;15.9	1	10.0	4	16.3±2.9		
CD	F	0	4	4.8±0.2**	4	4.8±0.7			2	4.1; 6.1			6	4.4±0.5
		10	4	6.6±0.6***	4	6.8±1.0***	2	16.3;15.2	2	9.8;11.4	4	20.6±6.6		
CD	S	0	4	4.8±0.2**	5	4.9±0.3*			2	4.1; 4.5			6	5.2±0.8*
		10	4	7.0±0.9***	4	8.1±1.1***	2	13.7;10.5			4	17.2±7.2		

Abbreviations as in Table 12.
1. Significance of the difference between treatment and control mean (= SSD + F + 0 mg dieldrin.kg^{-1}): *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

2. Dieldrin versus non-dieldrin statistical analysis (non-nodular livers)

	Exposure time (weeks)		
	15	52	68-72
SSD + F : 0 $mg.kg^{-1}$ v 10 $mg.kg^{-1}$	***	**	**
SSD + S : 0 $mg.kg^{-1}$ v 10 $mg.kg^{-1}$	***	**	
CD + F : 0 $mg.kg^{-1}$ v 10 $mg.kg^{-1}$	***	*	
CD + S : 0 $mg.kg^{-1}$ v 10 $mg.kg^{-1}$	**	***	

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; n.s.: not significant ($p > 0.05$).

3. Number of individual observations.
4. Mean ± standard deviation.

Table 18. Liver DNA concentration (non-nodular tissue)[1,2]. The results are expressed in mg liver DNA.g^{-1} liver.

Treatments			Exposure time (weeks)							
diet	bedding	dieldrin (mg.kg^{-1} diet)	n[3]	15	n	52	n	68-72	n	92
SSD	F	0	4	3.03±0.21[4]	4	2.59±0.26	3	2.62±0.09	6	2.66±0.13
		10	4	2.50±0.15**	6	2.39±0.22	8	2.73±0.15		
SSD	S	0	4	2.76±0.19	5	2.59±0.11	3	2.79±0.06*	6	3.00±0.21**
		10	4	2.28±0.05***	6	2.53±0.09	5	2.41±0.11*		
CD	F	0	4	2.70±0.15*	4	2.59±0.28	2	2.83;3.00*	6	2.59±0.17
		10	4	2.60±0.13*	6	2.82±0.34	6	2.59±0.14		
CD	S	0	4	2.60±0.20*	5	2.58±0.14	2	3.05;2.85*	6	2.77±0.32
		10	4	2.47±0.32*	6	2.28±0.27	4	2.53±0.19		

Abbreviations as in Table 12.

1. Significance of the difference between treatment and control mean (= SSD + F + 0 mg dieldrin.kg^{-1}): *p < 0.05; **p < 0.01; ***p < 0.001.

2. Dieldrin versus non-dieldrin statistical analysis:

	Exposure time (weeks)		
	15	52	68-72
SSD + F : 0 mg.kg^{-1} v 10 mg.kg^{-1}	**	n.s.	n.s.
SSD + S : 0 mg.kg^{-1} v 10 mg.kg^{-1}	**	n.s.	**
CD + F : 0 mg.kg^{-1} v 10 mg.kg^{-1}	n.s.	n.s.	*
CD + S : 0 mg.kg^{-1} v 10 mg.kg^{-1}	n.s.	*	*

*p < 0.05; **p < 0.01; ***p < 0.001; n.s.: not significant (p > 0.05).

3. Number of individual observations.

4. Mean ± standard deviation.

Table 19. Total liver DNA in non-nodular livers[1,2]. The results are expressed in mg liver DNA.100 g^{-1} bodyweight.

Treatments			Exposure time (weeks)							
diet	bedding	dieldrin (mg.kg^{-1} diet)	n[3]	15	n	52	n	68-72	n	92
SSD	F	0	4	12.8±0.5[4]	4	10.4±0.6	3	12.7± 1.3	6	11.1±0.7
		10	4	16.1±0.9***	6	15.3±2.3**	5	24.0± 3.4**		
SSD	S	0	4	12.6±0.6	5	10.9±0.7	3	11.3± 0.7	6	15.3±1.9**
		10	4	16.4±0.7***	4	19.7±4.7**	1	23.4		
CD	F	0	4	13.0±0.5	4	12.3±1.3*	2	11.6;18.5	6	10.7±0.7
		10	4	17.1±1.0***	4	18.4±3.6**	2	23.4;22.1		
CD	S	0	4	12.5±1.0	5	12.7±0.9**	2	12.9;12.4	6	14.4±2.2*
		10	4	17.1±2.5*	4	18.3±4.4**				

Abbreviations as in Table 12.

1. Significance of the difference between treatment and control mean (=SSD + F + 0 mg dieldrin. kg^{-1}): *p < 0.05; **p < 0.01; ***p < 0.001.

2. Dieldrin versus non-dieldrin statistical analysis:

	Exposure time (weeks)		
	15	52	68-72
SSD + F : 0 mg.kg^{-1} v 10 mg.kg^{-1}	***	***	**
SSD + S : 0 mg.kg^{-1} v 10 mg.kg^{-1}	***	**	
CD + F : 0 mg.kg^{-1} v 10 mg.kg^{-1}	***	*	
CD + S : 0 mg.kg^{-1} v 10 mg.kg^{-1}	*	*	

*p < 0.05; **p < 0.01;***p < 0.001; n.s.: not significant (p > 0.05).

3. Number of individual observations.

4. Mean ± standard deviation.

Table 20. Total liver DNA in nodular livers. The results are expressed in mg.100 g^{-1} bodyweight.

Treatments			Exposure time (weeks)							
			52				68-72			
diet	bedding	dieldrin (mg.kg^{-1} diet)	n[1]	Total liver DNA	Total non-nodular liver DNA	Total nodular liver DNA	n	Total liver DNA	Total non-nodular liver DNA	Total nodular liver DNA
SSD	F	10					3	33.2± 0.2[2]	22.9±2.5	10.4± 2.3
SSD	S	10	2	38.0[3] 45.9	17.2 15.7	20.8 30.2	4	38.4± 5.6	22.8±3.7	15.6± 8.0
CD	F	10	2	37.2 49.6	21.1 19.5	16.1 30.1	4	45.1±13.0	21.1±5.6	24.0±14.9
CD	S	10	2	24.2 34.6	14.4 15.5	9.8 19.1	4	43.0±18.0	22.4±4.7	20.6±16.1

Abbreviations as in Table 12.
1. Number of individual observations.
2. Mean ± standard deviation.
3. Individual results.

Table 21. Total liver protein (non-nodular tissue)[1,2]. The results are expressed in mg protein. g^{-1} liver.

Treatments			Exposure time (weeks)							
diet	bedding	dieldrin ($mg.kg^{-1}$ diet)	n[3]	15	n	52	n	68-72	n	92
SSD	F	0	4	227.8± 8.2[4]	4	207.8±12.3	2	202.7;196.6	6	200.2±12.3
		10	4	225.4±11.1	6	207.0±10.3	8	212.1± 8.8		
SSD	S	0	4	227.2±13.8	5	207.0±11.3	3	219.5± 2.9**	6	203.6±19.6
		10	4	212.8± 7.1	6	201.0±11.9	5	195.8± 9.8		
CD	F	0	4	226.2± 9.9	4	209.6± 8.3	2	245.5;256.9**	6	202.7±15.7
		10	4	221.2±14.1	6	208.9± 8.8	6	206.9± 10.7		
CD	S	0	4	210.4±19.6	5	203.8±11.5	2	242.6;226.4*	6	216.5± 9.8
		10	4	226.3±13.6	6	200.5±14.5	4	211.1± 13.9		

Abbreviations as in Table 12.

1. Significance of the difference between treatment and control mean (= SSD + F + 0 mg dieldrin. kg^{-1}): *p < 0.05; **p < 0.01; ***p < 0.001.

2. Dieldrin versus non-dieldrin statistical analysis:

	Exposure time (weeks)		
	15	52	68-72
SSD + F : 0 $mg.kg^{-1}$ v 10 $mg.kg^{-1}$	n.s.	n.s.	n.s.
SSD + S : 0 $mg.kg^{-1}$ v 10 $mg.kg^{-1}$	n.s.	n.s.	**
CD + F : 0 $mg.kg^{-1}$ v 10 $mg.kg^{-1}$	n.s.	n.s.	**
CD + S : 0 $mg.kg^{-1}$ v 10 $mg.kg^{-1}$	n.s.	n.s.	n.s.

*p < 0.05;**p < 0.01; ***p < 0.001; n.s.: not significant (p > 0.05).

3. Number of individual observations.

4. Mean ± standard deviation.

Table 22. Liver microsomal protein (non-nodular tissue)[1,2]. The results are expressed in mg protein.g^{-1} liver.

Treatments			Exposure time (weeks)							
diet	bedding	dieldrin (mg.kg^{-1} diet)	n[3]	15	n	52	n	68-72	n	92
SSD	F	0	4	98.5± 4.8[4]	4	84.2±5.8	2	96.8; 91.8	6	75.1±10.3
		10	4	93.1± 2.6	6	87.4±3.8	8	94.5± 7.5		
SSD	S	0	4	94.0± 6.6	5	82.5±2.3	3	84.5± 15.4	6	75.6± 7.2
		10	4	101.3± 7.2	6	88.6±6.9	5	85.0± 6.4		
CD	F	0	4	99.0± 4.4	4	83.7±3.3	2	99.2;114.7	6	81.5± 5.3
		10	4	107.1± 8.0	6	94.6±1.9**	6	93.8± 8.0		
CD	S	0	4	97.3±11.3	5	87.7±6.5	2	99.3; 81.8	6	86.6± 6.9*
		10	4	104.3±12.7	6	91.5±5.0	4	92.3± 9.9		

Abbreviations as in Table 12.

1. Significance of the difference between treatment and control mean (= SSD + F + 0 mg dieldrin . kg^{-1}): *p < 0.05; **p < 0.01; ***p < 0.001.

2. Dieldrin versus non-dieldrin statistical analysis:

	Exposure time (weeks)		
	15	52	68-72
SSD + F : 0 mg.kg^{-1} v 10 mg.kg^{-1}	n.s.	n.s.	n.s.
SSD + S : 0 mg.kg^{-1} v 10 mg.kg^{-1}	n.s.	n.s.	n.s.
CD + F : 0 mg.kg^{-1} v 10 mg.kg^{-1}	n.s.	***	n.s.
CD + S : 0 mg.kg^{-1} v 10 mg.kg^{-1}	n.s.	n.s.	n.s.

*p < 0.05; **p < 0.01; ***p < 0.001; n.s.: not significant (p > 0.05).

3. Number of individual observations.

4. Mean ± standard deviation.

Table 23. Liver soluble protein (non-nodular tissue)[1,2]. The results are expressed in mg protein.g^{-1} liver.

Treatments			Exposure time (weeks)							
diet	bedding	dieldrin (mg.kg^{-1} diet)	n[3]	15	n	52	n	68-72	n	92
SSD	F	0	4	71.4±2.0[4]	4	59.7±3.1	2	61.8;62.9	6	63.5±7.4
		10	4	69.4±4.0	5	61.7±2.9	6	66.4± 6.5		
SSD	S	0	4	70.8±1.8	4	63.2±2.9	3	65.3± 1.8	6	65.9±7.5
		10	4	70.3±4.8	5	63.9±1.5	5	60.5± 2.0		
CD	F	0	4	69.0±1.2	4	61.2±3.0	2	80.3;81.0*	6	65.8±4.4
		10	4	66.1±2.1	5	63.2±3.8	4	66.6± 4.3		
CD	S	0	4	66.6±6.0	5	60.4±2.5	2	66.3;68.1	6	66.3±4.1
		10	4	69.6±1.6	5	61.7±3.3	4	67.0± 3.9		

Abbreviations as in Table 12.
1. Significance of the difference between treatment and control mean (= SSD + F + 0 mg dieldrin .kg^{-1}): *p < 0.05; **p < 0.01; ***p < 0.001.

2. Dieldrin versus non-dieldrin statistical analysis:

	Exposure time (weeks)		
	15	52	68-72
SSD + F : 0 mg.kg^{-1} v 10 mg.kg^{-1}	n.s.	n.s.	n.s.
SSD + S : 0 mg.kg^{-1} v 10 mg.kg^{-1}	n.s.	n.s.	n.s.
CD + F : 0 mg.kg^{-1} v 10 mg.kg^{-1}	n.s.	n.s.	**
CD + S : 0 mg.kg^{-1} v 10 mg.kg^{-1}	n.s.	n.s.	n.s.

*p < 0.05; **p < 0.01; ***p < 0.001; n.s.: not significant (p > 0.05).

Table 24. Total liver protein/liver DNA quotients (non-nodular tissue)[1,2].

Treatments										
diet	bedding	dieldrin (mg.kg^{-1} diet)	n[3]	15	n	52	n	68-72	n	92
SSD	F	0	4	75.5±3.4[4]	4	81.6± 5.6	2	78.6;80.9	6	75.5±6.7
		10	4	90.4±6.2**	6	88.2± 6.8	8	77.9± 3.5		
SSD	S	0	4	82.3±2.5*	5	79.8± 1.8	3	78.7± 0.8	6	68.0±8.3
		10	4	93.4±2.8***	6	81.2± 4.6	5	81.6± 6.8		
CD	F	0	4	83.9±3.0*	4	81.8± 9.4	2	86.7;85.6*	6	78.9±6.4
		10	4	85.4±8.9	6	81.6± 9.8	6	80.1± 6.0		
CD	S	0	4	80.8±3.8	5	78.9± 2.0	2	79.5;79.4	6	78.9±7.6
		10	4	92.2±6.9**	6	90.3±13.6	4	83.5± 1.8		

Abbreviations as in Table 12.

1. Significance of the difference between treatment and control mean (= SSD + F + 0 mg dieldrin.kg^{-1}): *p < 0.05; **p < 0.01; ***p < 0.001.

2. Dieldrin versus non-dieldrin statistical analysis:

	Exposure time (weeks)		
	15	52	68-72
SSD + F : 0 mg.kg^{-1} v 10 mg.kg^{-1}	**	n.s.	n.s.
SSD + S : 0 mg.kg^{-1} v 10 mg.kg^{-1}	***	n.s.	n.s.
CD + F : 0 mg.kg^{-1} v 10 mg.kg^{-1}	n.s.	n.s.	n.s.
CD + S : 0 mg.kg^{-1} v 10 mg.kg^{-1}	*	n.s.	n.s.

*p < 0.05; **p < 0.01; ***p < 0.001; n.s.: not significant (p > 0.05).

3. Number of individual observations.

4. Mean ± standard deviation.

Table 25. Liver microsomal protein/liver DNA quotients (non-nodular tissue)[1,2].

Treatments			Exposure time (weeks)							
diet	bedding	dieldrin (mg.kg^{-1} diet)	n[3]	15	n	52	n	68-72	n	92
SSD	F	0	4	32.8±3.7[4]	4	33.1±3.2	2	35.6;35.6	6	28.4±4.8
		10	4	37.3±1.4	6	36.9±3.4	8	34.7± 2.3		
SSD	S	0	4	34.2±3.2	5	31.9±1.5	3	30.3± 5.1	6	25.3±3.9
		10	4	44.4±3.3**	6	35.8±3.2	4	35.4± 3.6		
CD	F	0	4	36.7±1.4	4	32.7±3.7	2	35.0;38.2	6	33.2±4.4
		10	4	41.6±4.1*	6	35.5±4.2	6	36.4± 4.0		
CD	S	0	4	37.5±4.6	5	34.0±2.5	2	32.6;28.7	6	31.6±4.0
		10	4	41.4±5.2*	6	41.1±4.7*				

Abbreviations as in Table 12.

1. Significance of the difference between treatment and control mean (= SSD + F + 0 mg dieldrin . kg^{-1}): *p < 0.05; **p < 0.01; ***p < 0.001).

2. Dieldrin versus non-dieldrin statistical analysis:

	Exposure time (weeks)		
	15	52	68-72
SSD + F : 0 mg.kg^{-1} v 10 mg.kg^{-1}	n.s.	n.s.	n.s.
SSD + S : 0 mg.kg^{-1} v 10 mg.kg^{-1}	**	*	n.s.
CD + F : 0 mg.kg^{-1} v 10 mg.kg^{-1}	n.s.	n.s.	n.s.
CD + S : 0 mg.kg^{-1} v 10 mg.kg^{-1}	n.s.	*	n.s.

*p < 0.05; **p < 0.01; ***p < 0.001; n.s.: not significant (p > 0.05).

3. Number of individual observations.

4. Mean ± standard deviation.

Table 26. Liver soluble protein/liver DNA quotients (non-nodular tissue)[1,2].

Treatments			Exposure time (weeks)							
diet	bedding	dieldrin ($mg.kg^{-1}$ diet)	n[3]	15	n	52	n	68-72	n	92
SSD	F	0	4	24.7±2.1[4]	4	23.4±1.6	2	22.7;24.4	6	24.0±3.0
		10	4	28.5±1.7*	5	26.2±2.3	6	24.6± 2.7		
SSD	S	0	4	26.7±0.4	4	24.3±1.4	3	23.4± 0.6	6	22.0±2.6
		10	4	30.8±2.2**	5	25.5±1.3	5	25.2± 1.3		
CD	F	0	4	26.0±1.7	4	23.9±2.6	2	28.4;27.0*	6	25.1±1.5
		10	4	25.2±1.8	5	23.9±2.2	4	26.1± 1.4		
CD	S	0	4	26.2±1.6	5	23.4±1.1	2	21.7;23.9	6	24.2±3.2
		10	4	29.0±0.8**	5	27.6±4.7	4	26.5± 0.5**		

Abbreviations as in Table 12.

1. Significance of the difference between treatment and control mean (= SSD + F + 0 mg dieldrin . kg^{-1}): *p < 0.05; **p < 0.01; ***p < 0.001.

2. Dieldrin versus non-dieldrin statistical analysis:

	Exposure time (weeks)		
	15	52	68-72
SSD + F : 0 $mg.kg^{-1}$ v 10 $mg.kg^{-1}$	*	n.s.	n.s.
SSD + S : 0 $mg.kg^{-1}$ v 10 $mg.kg^{-1}$	**	n.s.	n.s.
CD + F : 0 $mg.kg^{-1}$ v 10 $mg.kg^{-1}$	n.s.	n.s.	n.s.
CD + S : 0 $mg.kg^{-1}$ v 10 $mg.kg^{-1}$	*	n.s.	**

*p < 0.05; **p < 0.01; ***p < 0.001; n.s.: not significant (p < 0.05).

3. Number of individual observations.

4. Mean ± standard deviation.

Table 27. Liver *p*-nitroanisole *O*-demethylase specific activity (non-nodular tissue)[1,2]. The results are expressed in nmol *p*-nitrophenol formed.mg^{-1} microsomal protein.min.$^{-1}$.

Treatments			Exposure time (weeks)							
diet	bedding	dieldrin (mg.kg^{-1} diet)	n[3]	15	n	52	n	68-72	n	92
SSD	F	0	4	0.82±0.10[4]	4	0.58±0.06	2	0.65;1.06	6	1.13±0.37
		10	4	6.25±0.56***	6	4.90±0.82***	8	5.50±1.07***		
SSD	S	0	4	3.01±0.44**	5	1.90±0.40**	3	1.95±0.38*	6	1.53±0.58
		10	4	6.94±0.76***	6	4.96±0.68***	5	5.84±0.52***		
CD	F	0	4	1.62±0.41*	4	1.38±0.25**	2	1.19;1.26	6	2.14±0.56**
		10	4	4.94±0.47***	6	3.70±0.44***	6	4.62±0.92***		
CD	S	0	4	1.91±0.52*	5	1.57±0.42**	2	1.70;1.80*	6	2.64±0.73***
		10	4	4.87±1.02***	6	4.18±0.53***	4	4.98±0.81**		

Abbreviations as in Table 12.

1. Significance of the difference between treatment and control mean (= SSD + F + 0 mg dieldrin.kg^{-1}): *p < 0.05; **p < 0.01; ***p < 0.001.

2. Dieldrin versus non-dieldrin statistical analysis:

	Exposure time (weeks)		
	15	52	68-72
SSD + F : 0 mg.kg^{-1} v 10 mg.kg^{-1}	***	***	***
SSD + S : 0 mg.kg^{-1} v 10 mg.kg^{-1}	***	***	***
CD + F : 0 mg.kg^{-1} v 10 mg.kg^{-1}	***	***	**
CD + S : 0 mg.kg^{-1} v 10 mg.kg^{-1}	**	***	**

*p < 0.05; **p < 0.01; ***p < 0.001; n.s.: not significant (p > 0.05).

3. Number of individual observations.
4. Mean ± standard deviation.

Table 28. Liver epoxide hydratase specific activity (non-nodular tissue)[1,2]. The results are expressed in nmol styrene glycol formed.mg^{-1} microsomal protein.min.$^{-1}$.

Treatments			Exposure time (weeks)							
diet	bedding	dieldrin (mg.kg^{-1} diet)	n[3]	15	n	52	n	68-72	n	92
SSD	F	0	4	1.51±0.14[4]	4	1.12±0.08	2	1.20;1.20	6	1.75±0.48
		10	4	2.82±0.80*	6	3.12±0.59***	8	4.10±0.67***		
SSD	S	0	4	1.83±0.51	5	1.54±0.22*	2	2.60;1.56	6	1.50±0.44
		10	4	1.87±0.28	6	3.87±1.08**	5	4.58±0.33***		
CD	F	0	4	1.52±0.22	4	2.41±0.35**	2	1.78;2.22*	6	2.11±0.49
		10	4	2.76±0.32**	6	3.53±1.09**	3	4.56±0.87**		
CD	S	0	4	1.67±0.25	5	1.93±0.49*	2	1.45;2.76	6	2.33±0.64
		10	4	2.97±0.47**	6	3.10±0.86**	2	4.70;4.37***		

Abbreviations as in Table 12.

1. Significance of the difference between treatment and control mean (= SSD + F = 0 mg dieldrin. kg^{-1}): *p < 0.05; **p < 0.01; ***p < 0.001.

2. Dieldrin versus non-dieldrin statistical analysis:

	Exposure time (weeks)		
	15	52	68-72
SSD + F : 0 mg.kg^{-1} v 10 mg.kg^{-1}	*	***	***
SSD + S : 0 mg.kg^{-1} v 10 mg.kg^{-1}	n.s.	**	***
CD + F : 0 mg.kg^{-1} v 10 mg.kg^{-1}	***	n.s.	*
CD + S : 0 mg.kg^{-1} v 10 mg.kg^{-1}	**	*	*

*p < 0.05; **p < 0.01; ***p < 0.001; n.s.: not significant (p > 0.05).

3. Number individual observations.

4. Mean ± standard deviation.

Table 29. Liver glutathione *S*-epoxide transferase specific activity (non-nodular tissue)[1,2]. The results are expressed in µmol conjugated styrene oxide.mg^{-1} soluble protein.min.$^{-1}$.

Treatments			Exposure time (weeks)							
diet	bedding	dieldrin (mg.kg^{-1} diet)	n[3]	15	n	52	n	68-72	n	92
SSD	F	0	4	0.281±0.012[4]	4	0.339±0.016	2	0.275;0.330	6	0.343±0.018
		10	4	0.617±0.032***	5	0.665±0.101***	6	0.551±0.150*		
SSD	S	0	4	0.387±0.066*	4	0.357±0.039	3	0.378±0.018**	6	0.383±0.30*
		10	4	0.660±0.077***	5	0.718±0.112***	5	0.654±0.085***		
CD	F	0	4	0.333±0.008***	4	0.343±0.022	2	0.035;0.305	6	0.339±0.034
		10	4	0.634±0.166**	5	0.620±0.126**	4	0.612±0.079***		
CD	S	0	4	0.360±0.070	5	0.392±0.075	2	0.277;0.376	6	0.377±0.021*
		10	4	0.638±0.098***	5	0.637±0.077***	4	0.672±0.080***		

Abbreviations as in Table 12.

1. Significance of the difference between treatment and control mean (= SSD + F + 0 mg dieldrin.kg^{-1}): *p < 0.05; **p < 0.01; ***p < 0.001.

2. Dieldrin versus non-dieldrin statistical analysis:

	Exposure time (weeks)		
	15	52	68-72
SSD + F : 0 mg.kg^{-1} v 10 mg.kg^{-1}	***	***	*
SSD + S : 0 mg.kg^{-1} v 10 mg.kg^{-1}	**	***	**
CD + F : 0 mg.kg^{-1} v 10 mg.kg^{-1}	**	**	**
CD + S : 0 mg.kg^{-1} v 10 mg.kg^{-1}	**	***	**

*p < 0.05; **p < 0.01; ***p < 0.001; n.s.: not significant (p > 0.05).

3. Number of individual observations.

4. Mean ± standard deviation.

Table 30. Liver UDP-glucuronyl transferase specific activity (non-nodular tissue)[1,2]. The results are expressed in nmol *p*-nitrophenol conjugated.mg^{-1} microsomal protein.min^{-1}.

Treatments			Exposure time (weeks)							
diet	bedding	dieldrin ($mg.kg^{-1}$ diet)	n[3]	15	n	52	n	68-72	n	92
SSD	F	0	4	1.08±0.13[4]	3	1.61±0.13	2	1.74;1.86	6	1.48±0.10
		10	4	1.60±0.16*	5	2.17±0.19**	5	2.50±0.31**		
SSD	S	0	4	1.18±0.28	4	1.70±0.16	2	1.52;1.14*	6	1.35±0.26
		10	4	1.59±0.04**	5	1.99±0.10**	5	2.27±0.14**		
CD	F	0	4	1.41±0.19	3	1.70±0.17	2	1.53;1.54	6	1.48±0.12
		10	4	1.65±0.02**	5	1.89±0.12*	5	2.37±0.48		
CD	S	0	4	1.16±0.40	4	1.63±0.05	2	1.87;1.16	6	1.47±0.19
		10	4	1.66±0.03**	5	2.08±0.10**	4	2.54±0.50		

Abbreviations as in Table 12.

1. Significance of the difference between treatment and control mean (= SSD + F + 0 mg dieldrin.kg^{-1}): *p < 0.05; **p < 0.01; ***p < 0.001.

2. Dieldrin versus non-dieldrin statistical analysis:

	Exposure time (weeks)		
	15	52	68-72
SSD + F : 0 mg.kg^{-1} v 10 mg.kg^{-1}	**	**	*
SSD + S : 0 mg.kg^{-1} v 10 mg.kg^{-1}	n.s.	*	***
CD + F : 0 mg.kg^{-1} v 10 mg.kg^{-1}	n.s.	n.s.	n.s.
CD + S : 0 mg.kg^{-1} v 10 mg.kg^{-1}	n.s.	***	n.s.

*p < 0.05; **p < 0.01; ***p < 0.001; n.s.: not significant (p > 0.05).

3. Number of individual observations.

4. Mean ± standard deviation.

Table 31. Liver *p*-nitroanisole *O*-demethylase activity per unit DNA (non-nodular tissue)[1,2]. The results are expressed in nmol *p*-nitrophenol formed.mg^{-1} liver DNA.min^{-1}.

Treatments			Exposure time (weeks)							
diet	bedding	dieldrin (mg.kg^{-1} diet)	n[3]	15	n	52	n	68-72	n	92
SSD	F	0	4	26.6± 2.6[4]	4	18.8± 0.5	2	23.1;37.7	6	31.4±10.7
		10	4	233.4±27.7***	6	180.4±32.3***	8	199.9±50.3**		
SSD	S	0	4	105.9±25.9**	5	60.9±15.0**	3	58.8±12.9	6	38.1±14.9
		10	4	309.6±52.2***	6	178.4±35.5***	5	207.3±31.3***		
CD	F	0	4	59.2±13.2**	4	45.4±12.0*	2	41.7;48.2	6	69.3±14.0***
		10	4	204.1±29.6***	6	133.5±23.6***	6	178.0±48.8**		
CD	S	0	4	69.9±14.6**	5	52.7±11.5**	2	55.3;51.5	6	83.3±23.5***
		10	4	222.0±59.6***	6	170.3±21.1***	4	183.3±30.4**		

Abbreviations as in Table 12.

1. Significance of the difference between treatment and control mean (= SSD + F + 0 mg dieldrin.kg^{-1}): *p < 0.05; **p < 0.01; ***p < 0.001.

2. Dieldrin versus non-dieldrin statistical analysis:

	Exposure time (weeks)		
	15	52	68-72
SSD + F : 0 mg.kg^{-1} v 10 mg.kg^{-1}	***	***	**
SSD + S : 0 mg.kg^{-1} v 10 mg.kg^{-1}	***	***	***
CD + F : 0 mg.kg^{-1} v 10 mg.kg^{-1}	***	***	**
CD + S : 0 mg.kg^{-1} v 10 mg.kg^{-1}	**	***	***

*p < 0.05; **p < 0.01; ***p < 0.001; n.s.: not significant (p > 0.05).

3. Number of individual observations.
4. Mean ± standard deviation.

Table 32. Liver epoxide hydratase activity per unit DNA (non-nodular tissue)[1,2]. The results are expressed in nmol styrene glycol formed.mg^{-1} liver DNA.min^{-1}.

Treatments			Exposure time (weeks)							
diet	bedding	dieldrin (mg.kg^{-1} diet)	n[3]	15	n	52	n	68-72	n	92
SSD	F	0	4	46.9± 6.7[4]	4	37.1± 2.0	2	42.7; 42.7	6	49.8±17.5
		10	4	106.7±30.0*	6	114.3±19.2***	8	142.3± 25.9***		
SSD	S	0	4	63.4±12.5	5	46.5± 5.5*	2	67.8; 56.1	6	37.2±10.4
		10	4	82.8± 8.7	6	139.3±42.7**	5	161.5± 10.5***		
CD	F	0	4	55.8± 7.8	4	79.6±18.2*	2	62.4; 84.9	6	68.9±11.9*
		10	4	114.3±20.3**	6	126.5±45.4*	3	155.1± 24.6**		
CD	S	0	4	62.9± 6.6*	5	65.1±14.3*	2	47.2; 79.2	6	73.9±20.0*
		10	4	127.4±32.0**	6	127.9±40.8*	2	163.9;148.7***		

Abbreviations as in Table 12.

1. Significance of the difference between treatment and control mean (= SSD + F + 0 mg dieldrin.kg^{-1}): *p < 0.05; **p < 0.01; ***p < 0.001.

2. Dieldrin versus non-dieldrin statistical analysis:

	Exposure time (weeks)		
	15	52	68-72
SSD + F : 0 mg.kg^{-1} v 10 mg.kg^{-1}	*	***	***
SSD + S : 0 mg.kg^{-1} v 10 mg.kg^{-1}	*	**	***
CD + F : 0 mg.kg^{-1} v 10 mg.kg^{-1}	**	n.s.	*
CD + S : 0 mg.kg^{-1} v 10 mg.kg^{-1}	**	**	*

*p < 0.05; **p < 0.01; ***p < 0.001; n.s.: not significant (p > 0.05).

3. Number of individual observations.

4. Mean ± standard deviation.

Table 33. Liver glutathione *S*-epoxide transferase activity per unit DNA (non-nodular tissue)[1,2]. The results are expressed in µmol conjugated styrene oxide.mg^{-1} liver DNA.min^{-1}.

Treatments			Exposure time (weeks)							
diet	bedding	dieldrin (mg.kg^{-1} diet)	n[3]	15	n	52	n	68-72	n	92
SSD	F	0	4	6.6±0.2[4]	4	7.9±0.6	2	6.4;7.0	6	8.8±0.9
		10	4	17.3±2.1***	5	17.4±2.9***	6	13.5±3.4*		
SSD	S	0	4	10.0±2.2*	4	8.7±1.0	3	8.9±0.3***	6	8.4±0.7
		10	4	20.4±3.2***	5	18.4±3.6***	5	16.4±1.8***		
CD	F	0	4	8.5±0.6***	4	8.2±1.0	2	6.6;8.2	6	9.0±1.0
		10	4	16.2±5.1	5	14.5±4.1*	4	16.0±2.4		
CD	S	0	4	9.2±1.8*	5	9.2±1.8	2	8.2;6.6	6	9.1±1.0
		10	4	18.0±3.2***	5	17.6±3.7***	4	17.8±1.8***		

Abbreviations as in Table 12.

1. Significance of the difference between treatment and control mean (= SSD + F + 0 mg dieldrin.kg^{-1}): *p < 0.05; **p < 0.01; ***p < 0.0001.

2. Dieldrin versus non-dieldrin statistical analysis:

	Exposure time (weeks)		
	15	52	6 8-72
SSD + F : 0 mg.kg^{-1} v 10 mg.kg^{-1}	***	***	***
SSD + S : 0 mg.kg^{-1} v 10 mg.kg^{-1}	**	***	***
CD + F : 0 mg.kg^{-1} v 10 mg.kg^{-1}	*	*	**
CD + S : 0 mg.kg^{-1} v 10 mg.kg^{-1}	**	**	***

*p < 0.05; **p < 0.01; ***p < 0.001; n.s.: not significant (p > 0.05).

3. Number of individual observations.

4. Mean ± standard deviation.

Table 34. Liver UDP-glucuronyl transferase activity per unit DNA (non-nodular tissue)[1,2]. The results are expressed in nmol *p*-nitrophenol conjugated.mg^{-1} liver DNA.min^{-1}.

Treatments			Exposure time (weeks)							
diet	bedding	dieldrin (mg.kg^{-1} diet)	n[3]	15	n	52	n	68-72	n	92
SSD	F	0	4	32.8± 5.0[4]	3	52.6± 5.6	2	63.3;61.9	6	41.6±5.3
		10	4	60.3± 8.1**	5	77.5±12.4*	8	87.2±15.8		
SSD	S	0	4	41.3±11.9	4	52.9± 2.7	2	47.8;45.7**	6	33.9±6.7*
		10	4	73.2± 4.4***	5	69.5± 8.7*	5	80.5±10.9		
CD	F	0	4	51.5± 5.0*	3	54.0± 9.0	2	53.6;58.9	6	45.8±2.0
		10	4	65.4± 1.7***	5	68.9± 9.6*	6	92.6±24.3		
CD	S	0	4	43.1± 9.4	4	56.4± 3.9	2	60.9;33.3	6	46.5±8.5
		10	4	67.6± 9.9**	5	86.2±13.0**	4	94.5±25.4		

Abbreviations as in Table 12.

1. Significance of the difference between treatment and control mean (= SSD + F + 0 mg dieldrin.kg^{-1}): *p < 0.05; **p < 0.01; ***p < 0.001.

2. Dieldrin versus non-dieldrin statistical analysis:

	Exposure time (weeks)		
	15	52	68-72
SSD + F : 0 mg.kg^{-1} v 10 mg.kg^{-1}	**	*	n.s.
SSD + S : 0 mg.kg^{-1} v 10 mg.kg^{-1}	**	*	**
CD + F : 0 mg.kg^{-1} v 10 mg.kg^{-1}	**	n.s.	n.s.
CD + S : 0 mg.kg^{-1} v 10 mg.kg^{-1}	*	**	n.s.

*p < 0.05; **p < 0.01; ***p < 0.001; n.s.: not significant (p > 0.05).

3. Number of individual observations.

4. Mean ± standard deviation.

Table 35. Liver glucose-6-phosphatase specific activity (non-nodular tissue)[1,2]. The results are expressed in µg at $P_i.mg^{-1}$ microsomal protein.min^{-1}.

Treatments			Exposure time (weeks)							
diet	bedding	dieldrin ($mg.kg^{-1}$ diet)	n[3]	15	n	52	n	68–72	n	92
SSD	F	0	4	0.264±0.023[4]	4	0.243±0.005	2	0.249;0.263	6	0.278±0.050
		10	4	0.247±0.018	6	0.203±0.021**	8	0.227±0.026		
SSD	S	0	4	0.250±0.016	5	0.243±0.014	3	0.272±0.014	6	0.208±0.038*
		10	4	0.207±0.020**	6	0.197±0.054	5	0.170±0.017***		
CD	F	0	4	0.265±0.018	4	0.259±0.026	2	0.262;0.271	6	0.268±0.017
		10	4	0.227±0.039	6	0.193±0.027**	6	0.223±0.027		
CD	S	0	4	0.264±0.017	5	0.252±0.034	2	0.259;0.263	6	0.276±0.044
		10	4	0.229±0.020	6	0.193±0.020**	4	0.211±0.009***		

Abbreviations as in Table 12.

1. Significance of the difference between treatment and control mean (= SSD + F + 0 mg dieldrin.kg^{-1}): *p < 0.05; **p < 0.01; ***p < 0.001.

2. Dieldrin versus non-dieldrin statistical analysis:

	Exposure time (weeks)		
	15	52	68–72
SSD + F : 0 $mg.kg^{-1}$ v 10 $mg.kg^{-1}$	n.s.	**	n.s.
SSD + S : 0 $mg.kg^{-1}$ v 10 $mg.kg^{-1}$	*	n.s.	***
CD + F : 0 $mg.kg^{-1}$ v 10 $mg.kg^{-1}$	n.s.	**	n.s.
CD + S : 0 $mg.kg^{-1}$ v 10 $mg.kg^{-1}$	*	**	***

*p < 0.05; **p < 0.01; ***p < 0.001; n.s.: not significant (p > 0.05).

3. Number of individual observations.

4. Mean ± standard deviation.

Table 36. Liver glucose-6-phosphatase activity per unit DNA (non-nodular tissue)[1,2]. The results are expressed in µg at P_i.mg^{-1} liver DNA.min^{-1}.

Treatments			Exposure time (weeks)							
diet	bedding	dieldrin (mg.kg^{-1} diet)	n[3]	15	n	52	n	68-72	n	92
SSD	F	0	4	8.6±0.5[4]	4	8.1±0.7	2	8.9; 9.3	6	7.7±0.9
		10	4	9.2±0.8	6	7.5±1.0	8	7.9± 1.0		
SSD	S	0	4	8.6±0.8	5	7.8±0.6	3	8.3± 1.7	6	5.1±0.5***
		10	4	9.2±0.4	6	7.0±1.5	5	6.0± 1.0**		
CD	F	0	4	9.7±0.3	4	8.5±1.3	2	9.2;10.4	6	8.9±1.2
		10	4	9.3±0.9	6	6.8±1.2	6	8.4± 0.8		
CD	S	0	4	9.9±0.7	5	8.6±1.4	2	7.4; 8.6	6	8.7±1.4
		10	4	9.7±1.1	6	7.9±1.0	4	7.8± 0.5		

Abbreviations as in Table 12.

1. Significance of the difference between treatment and control mean (= SSD + F + 0 mg dieldrin.kg^{-1}): *p < 0.05; **p < 0.01; ***p < 0.001.

2. Dieldrin versus non-dieldrin statistical analysis:

	Exposure time (weeks)		
	15	52	68-72
SSD + F : 0 mg.kg^{-1} v 10 mg.kg^{-1}	n.s.	n.s.	n.s.
SSD + S : 0 mg.kg^{-1} v 10 mg.kg^{-1}	n.s.	n.s.	*
CD + F : 0 mg.kg^{-1} v 10 mg.kg^{-1}	n.s.	n.s.	n.s.
CD + S : 0 mg.kg^{-1} v 10 mg.kg^{-1}	n.s.	n.s.	n.s.

*p < 0.05; **p < 0.01; ***p < 0.001; n.s.: not significant (p > 0.05).

3. Number of individual observations.

4. Mean ± standard deviation.

Table 37. Liver glutathione concentration (non-nodular tissue)[1,2]. The results are expressed in $\mu mol.g^{-1}$ liver.

Treatments			Exposure time (weeks)				
diet	bedding	dieldrin ($mg.kg^{-1}$ diet)	n[3]	52	n	68-72	n	92
SSD	F	0	4	9.9±0.2[4]	3	10.5± 0.2	6	10.9±0.5
		10	4	10.2±0.5	8	10.9± 0.9		
SSD	S	0	4	10.5±0.5	2	11.8± 0.2	6	10.7±0.8
		10	4	10.1±0.7	5	11.1± 1.2		
CD	F	0	4	9.9±0.5	2	9.9; 9.3	6	10.9±0.2
		10	4	9.8±0.7	6	9.9± 0.4		
CD	S	0	4	10.6±0.3	2	10.2;10.5	6	11.2±0.6
		10	4	10.3±0.3	4	10.3± 0.6		

Abbreviations as in Table 12.

1. Significance of the difference between treatment and control mean (= SSD + F + 0 mg dieldrin.kg^{-1}): *p < 0.05; **p < 0.01; ***p < 0.001.

2. Dieldrin versus non-dieldrin statistical analysis:

	Exposure time (weeks)	
	52	68-72
SSD + F : 0 $mg.kg^{-1}$ v 10 $mg.kg^{-1}$	n.s.	n.s.
SSD + S : 0 $mg.kg^{-1}$ v 10 $mg.kg^{-1}$	n.s.	n.s.
CD + F : 0 $mg.kg^{-1}$ v 10 $mg.kg^{-1}$	n.s.	n.s.
CD + S : 0 $mg.kg^{-1}$ v 10 $mg.kg^{-1}$	n.s.	n.s.

*p < 0.05; **p < 0.01; ***p < 0.001; n.s.: not significant (p > 0.05).

3. Number of individual observations.

4. Mean ± standard deviation.

Table 38. Liver glutathione concentration per unit DNA (non-nodular tissue)[1],[2]. The results are expressed in μmol.mg^{-1} liver DNA.

Treatments			Exposure time (weeks)				
diet	bedding	dieldrin (mg.kg^{-1} diet)	n[3]	52	n	68-72	n	92
SSD	F	0	4	4.1±0.3[4]	3	4.0±0.1	6	4.1±0.2
		10	4	4.1±0.3	8	4.0±0.5		
SSD	S	0	4	4.2±0.2	2	4.1;4.4	6	3.6±0.3**
		10	4	4.1±0.4	5	4.6±0.4		
CD	F	0	4	4.0±0.5	2	3.3;3.3	6	4.2±0.3
		10	4	3.8±0.5	6	3.9±0.3		
CD	S	0	4	4.0±0.1	2	3.8;4.0	6	4.1±0.4
		10	4	4.5±0.8	4	4.1±0.3		

Abbreviations as in Table 12.

1. Significance of the difference between treatment and control mean (= SSD + F + 0 mg dieldrin.kg^{-1}): *p < 0.05; **p < 0.01; ***p < 0.001.

2. Dieldrin versus non-dieldrin statistical analysis:

	Exposure time (weeks)	
	52	68-72
SSD + F : 0 mg.kg^{-1} v 10 mg.kg^{-1}	n.s.	n.s.
SSD + S : 0 mg.kg^{-1} v 10 mg.kg^{-1}	n.s.	n.s.
CD + F : 0 mg.kg^{-1} v 10 mg.kg^{-1}	n.s.	n.s.
CD + S : 0 mg.kg^{-1} v 10 mg.kg^{-1}	n.s.	n.s.

*p < 0.05; **p < 0.01; ***p < 0.001; n.s.: not significant (p > 0.05).

3. Number of individual observations.

4. Mean ± standard deviation.

Table 39. Liver dieldrin concentration. The results are expressed in µg dieldrin.g^{-1} liver.

Treatments			Exposure time										
			15				52					68–72	
diet	bedding	dieldrin ($mg.kg^{-1}$ diet)	n[1]	non-nodular livers	n	non-nodular livers	n	nodular livers: host tissue	nodular livers: nodular tissue	n	non-nodular livers	n	nodular livers: host tissue / nodular tissue
SSD	F	0	4	<0.01	4	<0.01				3	<0.01		
		10	4	14.2±3.2[2]	6	17.1±6.7				5	15.1±4.6	3	9.0±2.5 / 9.8±1.6
SSD	S	0	4	<0.01	5	<0.01				3	<0.01		
		10	4	14.7±3.1	4	15.9±2.2	2	8.3; 3.6	11.0; 6.9	1	10.7	4	8.5±4.3 / 9.0±1.7
CD	F	0	4	<0.01	4	<0.01				2	<0.01		
		10	4	13.7±2.1	4	19.2±9.3	2	9.3; 5.7	9.9; 7.1	1	8.5	4	8.5±3.2 / 9.5±3.6
CD	S	0	4	<0.01	5	<0.01				2	<0.01		
		10	4	11.0±1.9	4	11.7±1.7	2	9.0;10.2	6.3;10.2			4	9.3±4.4 / 8.7±2.6

Abbreviations as in Table 12.
1. Number of individual observations.
2. Mean ± standard deviation.

Table 40. Liver dieldrin concentration per unit DNA. The results are expressed in µg dieldrin.mg^{-1} liver DNA.

Treatments			Exposure time										
diet	bedding	dieldrin (mg.kg^{-1} time)	15			52				68-72			
			n[1]	non-nodular livers	n	non-nodular livers	n	nodular livers		n	non-nodular livers	n	nodular livers	
								host tissue	nodular tissue				host tissue	nodular tissue
SSD	F	0	4	<0.01	4	<0.01				3	<0.01	3	3.2±0.8	3.8±1.0
		10	4	5.6±0.9[2]	6	7.4±3.5				5	5.5±1.3			
SSD	S	0	4	<0.01	5	<0.01				3	<0.01	4	3.5±1.7	4.1±1.0
		10	4	6.4±1.3	4	6.3±0.6	2	3.3;2.4	4.3;3.1	1	4.6			
CD	F	0	4	<0.01	4	<0.01				2	<0.01	4	3.3±1.5	4.0±1.6
		10	4	5.3±1.1	4	7.2±3.4	2	3.1;2.4	3.2;2.8	1	3.4			
CD	S	0	4	<0.01	5	<0.01				2	<0.01	4	3.7±1.9	3.4±1.0
		10	4	4.5±1.0	4	5.4±1.5	2	3.7;4.4	2.4;4.4					

Abbreviations as in Table 12.
1. Number of individual observations.
2. Mean ± standard deviation.

Table 41. Total liver dieldrin. The results are expressed in µg.

Treatments			Exposure time							
			15		52			68-72		
diet	bedding	dieldrin ($mg.kg^{-1}$ diet)	n[1]	non-nodular livers	n	non-nodular livers	n	nodular livers	n	non-nodular livers	n	nodular livers
SSD	F	0	4	<0.01	4	<0.01			3	<0.01		
		10	4	32.3±6.9[2]	6	50.0±30.8			5	52.5±9.4	3	40.0± 8.0
SSD	S	0	4	<0.01	5	<0.01			3	<0.01		
		10	4	35.9±8.8	4	59.5±15.2	2	83.0;44.3	1	56.7	4	64.6±18.1
CD	F	0	4	<0.01	4	<0.01			2	<0.01		
		10	4	32.9±6.6	4	67.8±44.4	2	68.3;44.5	1	43.9	4	68.8±24.0
CD	S	0	4	<0.01	5	<0.01			2	<0.01		
		10	4	29.1±7.1	4	46.7±11.9	2	43.5;46.8			4	57.6± 7.4

Abbreviations as in Table 12.
1. Number of individual observations.
2. Mean ± standard deviation.

Table 42. DNA concentration in non-nodular and nodular liver tissue of dieldrin-treated CF-1 mice. The results are expressed in $mg.g^{-1}$ liver.

Treatments			Exposure time (weeks)	Non-nodular liver tissue	Nodular liver tissue	Percentage increase/ decrease(-) in nodular liver tissue	Significance[1] non-nodular versus nodular liver tissue
diet	bedding	dieldrin ($mg.kg^{-1}$ diet)					
SSD	F	10	68-72	2.75	2.47	-10.2	
				2.74	2.87	4.7	
				2.84	2.37	-16.5	n.s.
SSD	S	10	52	2.62	2.23	-14.9	
				2.49	2.56	2.8	n.s.
			68-72	2.30	2.43	5.7	
				2.36	2.41	2.1	
				2.46	2.13	-13.4	
				2.57	2.11	-17.9	n.s.
CD	F	10	52	3.04	2.52	-17.1	
				3.04	3.01	- 1.0	n.s.
			68-72	2.43	2.37	- 2.5	
				2.67	2.35	-12.0	
				2.50	2.24	-10.4	
				2.80	2.46	-12.1	n.s.
CD	S	10	52	2.32	2.30	- 0.9	
				2.42	2.62	8.3	n.s.
			68-72	2.77	2.42	-12.6	
				2.38	2.52	5.9	
				2.59	2.57	- 0.8	
				2.38	2.47	3.8	n.s.

1. $*p < 0.05$; $**p < 0.01$; n.s.: not significant ($p > 0.05$).

Table 43. Total protein concentration in non-nodular and nodular liver tissue of dieldrin-treated CF-1 mice. The results are expressed in mg protein.g^{-1} liver.

Treatments			Exposure time (weeks)	Non-nodular liver tissue	Nodular liver tissue	Percentage increase/ decrease(-) in nodular liver tissue	Significance[1] non-nodular versus nodular liver tissue
diet	bedding	dieldrin ($mg.kg^{-1}$ diet)					
SSD	F	10	68-72	219.6	223.6	1.8	
				204.7	211.5	3.3	
				219.6	204.9	- 6.7	n.s.
SSD	S	10	52	211.8	215.7	1.8	
				190.1	174.8	- 8.0	n.s.
			68-72	200.5	186.6	- 6.9	
				203.7	188.4	- 7.5	
				179.6	195.5	3.3	
				193.6	152.4	-21.3	n.s.
CD	F	10	52	210.0	199.7	- 4.9	
				223.4	208.2	- 6.8	n.s.
			68-72	216.1	208.9	- 3.3	
				197.9	198.4	0.3	
				213.4	187.4	-12.2	
				209.5	198.4	- 5.3	n.s.
CD	S	10	52	200.5	188.4	- 6.0	
				220.7	202.4	- 8.3	n.s.
			68-72	230.8	203.7	-11.7	
				204.0	221.6	8.6	
				210.5	209.6	- 0.4	
				199.1	196.0	- 1.6	n.s.

Abbreviations as in Table 12.
1. $^{*}p < 0.05$; $^{**}p < 0.01$; n.s.: not significant ($p > 0.05$).

Table 44. Microsomal protein concentration in non-nodular and nodular liver tissue of dieldrin-treated CF-1 mice. The results are expressed in mg protein.g^{-1} liver.

Treatments			Exposure time (weeks)	Non-nodular liver tissue	Nodular liver tissue	Percentage increase/ decrease(-) in nodular liver tissue	Significance[1] non-nodular versus nodular liver tissue
diet	bedding	dieldrin (mg.kg^{-1} diet)					
SSD	F	10	68-72	94.8	75.9	-19.9	
				84.1	86.8	3.2	
				98.8	76.6	-22.5	n.s.
SSD	S	10	52	94.0	102.7	9.3	
				82.7	72.3	-12.6	n.s.
			68-72	92.5	83.2	-10.1	
				81.5	76.0	- 6.7	
				75.9	73.8	- 2.8	
				87.4	64.7	-26.0	n.s.
CD	F	10	52	97.3	80.8	-17.0	
				92.7	89.2	- 3.8	n.s.
			68-72	107.2	69.8	-34.9	
				94.0	86.1	- 8.4	
				82.5	68.9	-16.5	
				95.8	91.8	- 4.2	n.s.
CD	S	10	52	86.9	79.8	- 8.2	
				98.4	88.4	-10.2	n.s.
			68-72	104.0	96.6	- 7.1	
				97.8	101.7	4.0	
				90.3	99.1	9.7	
				81.0	90.2	11.4	n.s.

Abbreviations as in Table 12.
1. $^{*}p < 0.05$; $^{**}p < 0.01$; n.s.: not significant ($p > 0.05$).

Table 45. Soluble protein concentration in non-nodular and nodular liver tissue of dieldrin-treated CF-1 mice. The results are expressed in mg protein.g^{-1} liver.

Treatments			Exposure time (weeks)	Non-nodular liver tissue	Nodular liver tissue	Percentage increase/ decrease(-) in nodular liver tissue	Significance[1] non-nodular versus nodular liver tissue
diet	bedding	dieldrin (mg.kg^{-1} diet)					
SSD	F	10	68-72	72.3	73.9	2.2	
SSD	S	10	52	65.6	59.2	- 9.8	
			68-72	62.7	60.5	- 3.5	
				59.0	63.0	6.8	
				58.7	61.3	4.4	
				57.3	58.1	1.4	n.s.
CD	F	10	52	67.8	63.3	- 6.6	
			68-72	65.8	63.4	- 3.6	
				65.8	61.2	- 7.0	n.s.
CD	S	10	52	56.0	65.6	17.1	
			68-72	71.4	68.6	- 3.9	
				63.4	68.7	8.4	
				69.1	60.4	-12.6	
				64.0	65.2	1.9	n.s.

Abbreviations as in Table 12.
1. *$p < 0.05$; **$p < 0.01$; n.s.: not significant ($p > 0.05$).

Table 46. Total protein/DNA quotients in non-nodular and nodular liver tissue of dieldrin-treated CF-1 mice.

Treatments			Exposure time (weeks)	Non-nodular liver tissue	Nodular liver tissue	Percentage increase/ decrease(-) in nodular liver tissue	Significance[1] non-nodular versus nodular liver tissue
diet	bedding	dieldrin ($mg.kg^{-1}$ diet)					
SSD	F	10	68-72	79.8	90.5	13.4	
				74.7	73.7	- 1.3	
				77.3	86.5	11.9	n.s.
SSD	S	10	52	85.1	84.3	- 0.9	
				81.9	78.4	- 4.3	n.s.
			68-72	87.2	76.8	-11.9	
				86.3	78.2	- 9.4	
				73.0	87.1	19.3	
				75.3	80.2	6.5	n.s.
CD	F	10	52	69.0	65.7	- 4.8	
				93.1	82.6	-11.3	n.s.
			68-72	88.9	88.1	- 0.9	
				74.1	84.4	13.9	
				85.4	83.7	- 2.0	
				74.8	80.7	- 7.9	n.s.
CD	S	10	52	82.8	83.5	1.5	
				103.1	93.3	- 9.5	n.s.
			68-72	83.3	84.2	1.1	
				85.7	87.9	2.6	
				81.3	81.6	0.4	
				83.6	79.4	- 5.0	n.s.

Abbreviations as in Table 12.
1. $^{*}p < 0.05$; $^{**}p < 0.01$; n.s.: not significant ($p > 0.05$).

Table 47. Microsomal protein/DNA quotients in non-nodular and nodular liver tissue of dieldrin-treated CF-1 mice.

Treatments			Exposure time (weeks)	Non-nodular liver tissue	Nodular liver tissue	Percentage increase/ decrease(-) in nodular liver tissue	Significance[1] non-nodular versus nodular liver tissue
diet	bedding	dieldrin ($mg.kg^{-1}$ diet)					
SSD	F	10	68-72	34.4	30.7	-10.8	
				30.7	30.2	- 1.6	
				34.8	32.3	- 7.2	n.s.
SSD	S	10	52	37.8	40.1	6.1	
				35.6	32.4	- 9.0	n.s.
			68-72	40.2	32.6	-18.9	
				34.5	31.5	- 8.7	
				30.8	34.6	12.3	
				34.0	34.0	0	n.s.
CD	F	10	52	32.0	29.6	- 7.5	
				38.6	35.4	- 8.3	n.s.
			68-72	44.1	29.5	-33.1	
				35.2	36.6	4.0	
				33.0	30.8	- 6.7	
				34.2	37.3	9.1	n.s.
CD	S	10	52	35.9	30.5	-15.0	
				46.0	40.7	-11.5	n.s.
			68-72	37.5	39.9	6.4	
				41.1	42.2	2.7	
				34.9	38.6	10.6	
				34.0	36.5	7.4	*

Abbreviations as in Table 12.
1. $^{*}p < 0.05$; $^{**}p < 0.01$; n.s.: not significant ($p > 0.05$).

Table 48. Soluble protein/DNA quotients in non-nodular and nodular liver tissue of dieldrin-treated CF-1 mice.

Treatments			Exposure time (weeks)	Non-nodular liver tissue	Nodular liver tissue	Percentage increase/ decrease(-) in nodular liver tissue	Significance[1] non-nodular versus nodular liver tissue
diet	bedding	dieldrin ($mg.kg^{-1}$ diet)					
SSD	F	10	68-72	26.3	29.9	13.7	
SSD	S	10	52	26.3	23.1	-12.2	
			68-72	27.3	24.9	- 8.8	
				25.0	26.1	4.4	
				23.9	28.8	20.5	
				24.4	28.0	14.8	n.s.
CD	F	10	52	22.3	20.8	- 6.7	
			68-72	27.1	26.8	- 1.1	
				24.6	26.0	5.7	n.s.
CD	S	10	52	23.1	25.0	8.2	
			68-72	25.8	28.3	9.7	
				26.6	28.5	7.1	
				26.7	23.5	-12.0	
				26.9	26.4	- 1.9	n.s.

Abbreviations as in Table 12.
1. $^{*}p < 0.05$; $^{**}p < 0.01$; n.s.: not significant ($p > 0.05$).

Table 49. *p*-Nitroanisole *O*-demethylase specific activity in non-nodular and nodular liver tissue of dieldrin-treated CF-1 mice. The results are expressed in nmol *p*-nitrophenol formed.mg^{-1} microsomal protein.min^{-1}.

Treatments			Exposure time (weeks)	Non-nodular liver tissue	Nodular liver tissue	Percentage increase/ decrease(-) in nodular liver tissue	Significance[1] non-nodular versus nodular liver tissue
diet	bedding	dieldrin ($mg.kg^{-1}$ diet)					
SSD	F	10	68-72	3.94	6.76	71.6	
				5.56	7.84	41.0	
				6.57	8.18	24.5	*
SSD	S	10	52	5.52	6.65	20.5	
				4.15	5.85	41.0	n.s.
			68-72	5.76	6.33	9.9	
				5.10	7.00	37.3	
				5.75	7.35	27.8	
				6.10	8.66	42.0	*
CD	F	10	52	3.59	5.13	42.9	
				3.05	5.90	93.4	n.s.
			68-72	5.53	8.14	47.2	
				3.09	6.32	104.5	
				5.13	8.37	63.2	
				4.63	6.87	48.4	**
CD	S	10	52	4.38	5.57	27.2	
				3.18	6.07	90.9	n.s.
			68-72	5.83	8.35	43.2	
				4.11	7.39	79.8	
				5.48	8.29	51.3	
				4.48	7.03	56.9	**

Abbreviations as in Table 12.
1. $^{*}p < 0.05$; $^{**}p < 0.01$; n.s.: not significant ($p > 0.05$).

Table 50. Epoxide hydratase specific activity in non-nodular and nodular liver tissue of dieldrin-treated CF-1 mice. The results are expressed in nmol styrene glycol formed. mg^{-1} microsomal protein.min^{-1}.

Treatments			Exposure time (weeks)	Non-nodular liver tissue	Nodular liver tissue	Percentage increase/ decrease(-) in nodular liver tissue	Significance[1] non-nodular versus nodular liver tissue
diet	bedding	dieldrin ($mg.kg^{-1}$ diet)					
SSD	F	10	68-72	3.22	4.72	46.6	
				3.59	6.09	69.6	
				3.32	5.38	62.0	n.s.
SSD	S	10	52	4.94	5.10	3.2	
				4.65	7.73	66.2	n.s.
			68-72	4.40	6.20	40.9	
				4.30	6.40	48.8	
				5.10	5.80	13.7	
				4.70	5.05	7.4	n.s.
CD	F	10	52	2.64	3.90	47.7	
				3.51	5.76	64.1	n.s.
			68-72	4.05	6.39	57.8	
				5.56	5.91	6.3	n.s.
CD	S	10	52	2.42	5.14	112.4	
				2.02	4.91	143.1	n.s.
			68-72	4.70	5.90	25.5	
				4.37	5.55	27.0	n.s.

Abbreviations as in Table 12.
1. $^{*}p < 0.05$; $^{**}p < 0.01$; n.s.: not significant ($p > 0.05$).

Table 51. Glutathione *S*-epoxide transferase specific activity in non-nodular and nodular liver tissue of dieldrin-treated CF-1 mice. The results are expressed in µmol conjugated styrene oxide.mg^{-1} soluble protein.min^{-1}.

Treatments			Exposure time (weeks)	Non-nodular liver tissue	Nodular liver tissue	Percentage increase/ decrease(-) in nodular liver tissue	Significance[1] non-nodular versus nodular liver tissue
diet	bedding	dieldrin ($mg.kg^{-1}$ diet)					
SSD	F	10	68-72	0.441	0.559	26.8	
SSD	S	10	52	0.682	0.940	37.8	
			68-72	0.613	0.790	28.9	
				0.531	0.788	48.4	
				0.755	0.799	5.8	
				0.678	0.834	23.0	*
CD	F	10	52	0.495	0.705	42.4	
			68-72	0.656	0.801	22.1	
				0.506	0.619	22.3	n.s.
CD	S	10	52	0.678	0.860	26.8	
			68-72	0.773	0.918	18.8	
				0.700	0.831	18.7	
				0.605	0.706	16.7	
				0.612	0.682	11.4	**

Abbreviations as in Table 12.
1. $^{*}p < 0.05$; $^{**}p < 0.01$; n.s.: not significant ($p > 0.05$).

Table 52. UDP-glucuronyl transferase specific activity in non-nodular and nodular liver tissue of dieldrin-treated CF-1 mice. The results are expressed in nmol conjugated *p*-nitrophenol.mg^{-1} microsomal protein.min^{-1}.

Treatments			Exposure time (weeks)	Non-nodular liver tissue	Nodular liver tissue	Percentage increase/ decrease(-) in nodular liver tissue	Significance[1] non-nodular versus nodular liver tissue
diet	bedding	dieldrin ($mg.kg^{-1}$ diet)					
SSD	F	10	68-72	2.68	1.40	-47.8	
				1.86	2.46	32.3	
				2.46	1.73	-29.7	n.s.
SSD	S	10	52	2.10	2.36	12.4	
			68-72	2.41	2.73	13.3	
				2.32	2.66	14.7	
				2.15	1.55	-27.9	
				2.38	1.91	-19.7	n.s.
CD	F	10	52	1.72	2.08	20.9	
			68-72	2.19	1.88	-14.2	
				1.96	2.77	41.3	
				2.74	1.96	-28.5	
				1.94	2.68	38.1	n.s.
CD	S	10	52	2.06	2.42	17.5	
			68-72	3.03	2.80	- 7.6	
				2.88	1.72	-40.3	
				2.24	1.86	-17.0	
				1.99	2.59	30.2	n.s.

Abbreviations as in Table 12.
1. $^{*}p < 0.05$; $^{**}p < 0.01$; n.s.: not significant ($p > 0.05$).

Table 53. *p*-Nitroanisole *O*-demethylase activity per unit DNA in non-nodular and nodular liver tissue of dieldrin-treated CF-1 mice. The results are expressed in nmol *p*-nitrophenol formed.mg^{-1} liver DNA.min^{-1}.

Treatment			Exposure time (weeks)	Non-nodular liver tissue	Nodular liver tissue	Percentage increase/ decrease(-) in nodular liver tissue	Significance[1] non-nodular versus nodular liver tissue
diet	bedding	dieldrin ($mg.kg^{-1}$ diet)					
SSD	F	10	68-72	120.9	204.4	69.1	
				193.4	253.4	31.0	
				226.5	251.4	11.0	n.s.
SSD	S	10	52	208.4	266.8	28.0	
				147.9	189.7	28.3	n.s.
			68-72	177.7	219.3	23.4	
				173.4	297.7	71.7	
				231.2	251.7	8.9	
				210.7	273.1	29.6	n.s.
CD	F	10	52	114.9	136.4	18.7	
				117.8	208.8	77.2	n.s.
			68-72	243.3	250.4	2.9	
				105.7	235.8	123.1	
				180.6	306.7	69.8	
				204.2	202.3	- 0.9	n.s.
CD	S	10	52	157.3	169.7	7.9	
				144.2	247.3	71.5	n.s.
			68-72	203.3	322.0	58.4	
				139.9	300.9	115.1	
				205.7	330.9	60.9	
				184.1	296.6	61.1	**

Abbreviations as in Table 12.
1. $^{*}p < 0.05$; $^{**}p < 0.01$; n.s.: not significant ($p > 0.05$).

Table 54. Epoxide hydratase activity per unit DNA in non-nodular and nodular liver tissue of dieldrin-treated CF-1 mice. The results are expressed in nmol styrene glycol formed.mg^{-1} liver DNA.min^{-1}.

Treatments			Exposure time (weeks)	Non-nodular liver tissue	Nodular liver tissue	Percentage increase/ decrease(-) in nodular liver tissue	Significance[1] non-nodular versus nodular liver tissue
diet	bedding	dieldrin ($mg.kg^{-1}$ diet)					
SSD	F	10	68-72	111.0	145.0	30.6	
				110.2	184.2	67.2	
				115.5	173.9	50.6	*
SSD	S	10	52	186.5	204.6	9.7	
				165.7	250.6	51.2	n.s.
			68-72	177.0	212.3	19.9	
				148.5	201.8	35.9	
				157.3	201.3	28.0	
				159.8	171.9	7.6	*
CD	F	10	52	84.5	103.7	22.7	
				135.6	203.9	50.4	n.s.
			68-72	142.5	234.1	64.3	
				183.5	181.8	- 0.9	n.s.
CD	S	10	52	86.9	156.5	80.1	
				92.2	200.0	116.9	n.s.
			68-72	163.9	227.5	38.8	
				148.7	202.7	36.3	n.s.

Abbreviations as in Table 12.
1. $^{*}p < 0.05$; $^{**}p < 0.01$; n.s.: not significant ($p > 0.05$).

Table 55. Glutathione *S*-epoxide transferase activity per unit DNA in non-nodular and nodular liver tissue of dieldrin-treated CF-1 mice. The results are expressed in µmol conjugated styrene oxide.mg^{-1} liver DNA.min^{-1}.

Treatments			Exposure time (weeks)	Non-nodular liver tissue	Nodular liver tissue	Percentage increase/ decrease(-) in nodular liver tissue	Significance[1] non-nodular versus nodular liver tissue
diet	bedding	dieldrin ($mg.kg^{-1}$ diet)					
SSD	F	10	68-72	11.6	16.7	44.0	
SSD	S	10	52	18.0	21.7	20.5	
			68-72	16.7	19.7	18.0	
				13.3	20.6	54.9	
				18.0	23.0	27.8	
				16.5	21.0	27.3	n.s.
CD	F	10	52	11.0	14.7	33.6	
			68-72	17.8	21.4	20.2	
				12.5	16.1	28.8	n.s.
CD	S	10	52	15.7	21.5	36.9	
			68-72	19.9	26.0	30.7	
				18.6	23.7	27.4	
				16.1	16.6	3.1	
				16.5	18.0	9.1	n.s.

Abbreviations as in Table 12.
1. $^{*}p < 0.05$; $^{**}p < 0.01$; n.s.: not significant ($p > 0.05$).

Table 56. UDP-glucuronyl transferase activity per unit DNA in non-nodular and nodular liver tissue of dieldrin-treated CF-1 mice. The results are expressed in nmol conjugated *p*-nitrophenol.mg^{-1} liver DNA.min^{-1}.

Treatments			Exposure time (weeks)	Non-nodular liver tissue	Nodular liver tissue	Percentage increase/ decrease(-) in nodular liver tissue	Significance[1] non-nodular versus nodular liver tissue
diet	bedding	dieldrin ($mg.kg^{-1}$ diet)					
SSD	F	10	68-72	57.1	74.4	30.3	
				85.6	55.9	-34.7	
				92.4	43.0	-53.5	n.s.
SSD	S	10	52	79.3	94.7	19.4	
			68-72	66.3	53.7	19.0	
				80.9	81.2	0.4	
				96.9	93.5	- 3.5	
				80.1	93.9	4.7	n.s.
CD	F	10	52	55.1	55.3	0.4	
			68-72	120.6	60.3	-50.0	
				66.4	91.8	38.3	
				69.0	101.5	47.1	
				96.6	55.4	-42.7	n.s.
CD	S	10	52	74.0	73.7	- 0.4	
			68-72	78.1	71.7	- 8.2	
				67.7	105.5	55.8	
				113.8	111.8	- 1.8	
				118.4	72.6	-38.7	n.s.

Abbreviations as in Table 12.
1. $^{*}p < 0.05$; $^{**}p < 0.01$; n.s.: not significant ($p > 0.05$).

Table 57. Glucose-6-phosphatase specific activity in non-nodular and nodular liver tissue of dieldrin-treated CF-1 mice. The results are expressed in µg at P_i.mg^{-1} microsomal protein.min^{-1}.

Treatments			Exposure time (weeks)	Non-nodular liver tissue	Nodular liver tissue	Percentage increase/ decrease(-) in nodular liver tissue	Significance[1] non-nodular versus nodular liver tissue
diet	bedding	dieldrin ($mg.kg^{-1}$ diet)					
SSD	F	10	68-72	0.189	0.109	-42.3	
				0.249	0.089	-64.3	
				0.242	0.083	-65.7	*
SSD	S	10	52	0.150	0.075	-50.0	
				0.157	0.108	-31.2	n.s.
			68-72	0.168	0.102	-39.3	
				0.154	0.068	-55.8	
				0.164	0.092	-43.9	
				0.166	0.067	-59.6	**
CD	F	10	52	0.183	0.094	-48.6	
				0.156	0.089	-42.9	n.s.
			68-72	0.188	0.091	-51.6	
				0.198	0.098	-50.5	
				0.221	0.070	-68.3	
				0.258	0.094	-63.6	**
CD	S	10	52	0.199	0.095	-52.3	
				0.186	0.103	-44.6	n.s.
			68-72	0.201	0.078	-61.2	
				0.207	0.085	-58.9	
				0.212	0.101	-52.4	
				0.223	0.078	-65.0	**

Abbreviations as in Table 12.
1. *$p < 0.05$; **$p < 0.01$; n.s.: not significant ($p > 0.05$).

Table 58. Glucose-6-phosphatase activity per unit DNA in non-nodular and nodular liver tissue of dieldrin-treated CF-1 mice. The results are expressed in µg at P_i.mg^{-1} liver DNA.min^{-1}.

Treatments			Exposure time (weeks)	Non-nodular liver tissue	Nodular liver tissue	Percentage increase/ decrease(-) in nodular liver tissue	Significance[1] non-nodular versus nodular liver tissue
diet	bedding	dieldrin ($mg.kg^{-1}$ diet)					
SSD	F	10	68-72	6.5	3.4	-47.7	
				7.6	2.7	-64.5	
				8.4	2.7	-67.9	*
SSD	S	10	52	5.7	3.0	-47.4	
				5.6	3.5	-37.5	n.s.
			68-72	6.7	3.5	-47.8	
				5.3	2.1	-60.4	
				5.1	3.2	-37.3	
				5.6	2.8	-50.0	**
CD	F	10	52	5.9	2.5	-57.6	
				6.0	3.1	-48.3	n.s.
			68-72	7.3	2.7	-63.0	
				8.1	3.6	-55.6	
				9.7	2.1	-78.4	
				8.8	3.5	-60.2	**
CD	S	10	52	7.1	2.9	-59.2	
				8.5	4.2	-50.6	**
			68-72	7.5	3.1	-58.7	
				8.5	3.4	-60.0	
				7.4	3.9	-47.3	
				7.6	2.9	-61.8	**

Abbreviations as in Table 12.
1. *$p < 0.05$; **$p < 0.01$; n.s.: not significant ($p > 0.05$).

Table 59. Glutathione concentration in non-nodular and nodular liver tissue of dieldrin-treated CF-1 mice. The results are expressed in $\mu mol.g^{-1}$ liver.

Treatments			Exposure time (weeks)	Non-nodular liver tissue	Nodular liver tissue	Percentage increase/ decrease(-) in nodular liver tissue	Significance[1] non-nodular versus nodular liver tissue
diet	bedding	dieldrin ($mg.kg^{-1}$ diet)					
SSD	F	10	68-72	10.5	5.2	-50.5	
				11.0	7.7	-30.0	
				9.9	5.5	-44.4	*
SSD	S	10	68-72	12.8	6.4	-50.0	
				10.7	5.4	-49.5	
				10.8	5.4	-50.0	
				10.1	5.1	-49.5	**
CD	F	10	52	10.7	6.0	-43.9	
			68-72	10.4	6.7	-35.6	
				9.1	7.8	-14.3	
				10.1	5.7	-43.6	
				10.0	7.0	-30.0	*
CD	S	10	68-72	10.2	5.7	-44.1	
				9.9	7.3	-26.3	
				11.2	7.0	-37.5	
				10.0	6.3	-37.0	**

Abbreviations as in Table 12.
1. $^{*}p < 0.05$; $^{**}p < 0.01$; n.s.: not significant ($p > 0.05$).

Table 60. Glutathione/DNA quotients in non-nodular and nodular liver tissue of dieldrin-treated CF-1 mice. The results are expressed in $\mu mol.mg^{-1}$ liver DNA.

Treatments			Exposure time (weeks)	Non-nodular liver tissue	Nodular liver tissue	Percentage increase/ decrease(-) in nodular liver tissue	Significance[1] non-nodular versus nodular liver tissue
diet	bedding	dieldrin ($mg.kg^{-1}$ diet)					
SSD	F	10	68-72	3.8	2.1	-44.7	
				4.0	2.7	-32.5	**
				3.5	2.3	-34.3	
SSD	S	10	68-72	5.2	3.0	-42.3	
				4.2	2.8	-33.3	
				4.7	3.0	-36.2	
				4.4	2.4	-45.5	**
CD	F	10	52	3.5	2.0	-42.9	
			68-72	4.3	2.8	-34.9	
				3.8	3.3	-13.2	
				4.0	2.5	-37.5	*
				3.6	2.8	-22.2	
CD	S	10	68-72	3.7	2.3	-37.8	
				4.2	2.9	-31.0	
				4.3	2.7	-37.2	
				4.2	2.6	-38.1	**

Abbreviations as in Table 12.
1. $^{*}p < 0.05$; $^{**}p < 0.01$; n.s.: not significant ($p > 0.05$).

Table 61. Dieldrin concentration in non-nodular and nodular liver tissue of dieldrin-treated CF-1 mice. The results are expressed in µg dieldrin.g^{-1} liver.

Treatments			Exposure time (weeks)	Non-nodular liver tissue	Nodular liver tissue	Percentage increase/ decrease(-) in nodular liver tissue	Significance[1] non-nodular versus nodular liver tissue
diet	bedding	dieldrin ($mg.kg^{-1}$ diet)					
SSD	F	10	68-72	10.8	10.9	0.9	
				6.2	7.9	27.4	
				10.0	10.5	5.0	n.s.
SSD	S	10	52	8.3	11.0	32.5	
				6.4	6.9	7.8	n.s.
			68-72	6.3	8.7	38.1	
				5.3	7.7	45.3	
				14.8	11.6	-21.6	
				7.6	8.2	7.9	n.s.
CD	F	10	52	9.3	9.9	6.5	
				5.7	7.1	24.6	n.s.
			68-72	12.7	14.6	15.0	
				6.8	8.0	17.6	
				9.1	9.2	2.0	
				5.3	6.1	15.1	n.s.
CD	S	10	52	9.0	6.3	-30.0	
				10.2	10.2	0	n.s.
			68-72	6.8	6.9	1.5	
				14.6	11.8	-19.2	
				11.0	9.9	-10.0	
				4.8	6.2	29.2	n.s.

Abbreviations as in Table 12.
1. $^{*}p < 0.05$; $^{**}p < 0.01$; n.s.: not significant ($p > 0.05$).

Table 62. Dieldrin/DNA quotients in non-nodular and nodular liver tissue of dieldrin-treated CF-1 mice. The results are expressed in µg dieldrin.mg^{-1} liver DNA.

Treatments			Exposure time (weeks)	Non-nodular liver tissue	Nodular liver tissue	Percentage increase/ decrease(-) in nodular liver tissue	Significance[1] non-nodular versus nodular liver tissue
diet	bedding	dieldrin ($mg.kg^{-1}$ diet)					
SSD	F	10	68-72	3.9	4.4	12.8	
				2.3	2.7	17.4	
				3.5	4.4	25.7	n.s.
SSD	S	10	52	3.3	4.3	30.3	
				2.4	3.1	29.2	n.s.
			68-72	2.7	3.6	33.3	
				2.2	3.2	45.5	
				6.0	5.4	-10.0	
				3.0	4.3	43.3	n.s.
CD	F	10	52	3.1	3.2	3.2	
				2.4	2.8	16.7	n.s.
			68-72	5.2	6.2	19.2	
				2.5	3.4	36.0	
				3.6	4.1	13.9	
				1.9	2.5	31.6	*
CD	S	10	52	3.7	2.4	-35.1	
				4.4	4.4	0	n.s.
			68-72	2.5	2.8	12.0	
				6.2	4.7	-24.2	
				4.2	3.8	- 9.5	
				2.0	2.5	25.0	n.s.

Abbreviations as in Table 12.
1. $^{*}p < 0.05$; $^{**}p < 0.01$; n.s.: not significant ($p > 0.05$).

Table 63. Effect of dieldrin withdrawal on liver weight.

Dietary[1] dieldrin (mg.kg^{-1})	Age (weeks)	n[2]	Bodyweight (g)	Liver weight (g)			Relative liver weight (g.100 g^{-1} bodyweight)			Liver dieldrin residu levels (µg.g^{-1} liver)	
				non-nodular	nodular	total	non-nodular	nodular	total	non-nodular	nodular
0	92	6	44.7±2.9[3]			1.95±0.22			4.37±0.52	<0.01	
10	68-72		36.5	1.66	9.27	10.93	4.55	25.40	29.95		
			38.8	3.89	3.28	7.17	10.03	8.45	18.48		
			41.4	2.59	3.35	5.94	6.27	8.09	14.36		
			44.0	2.68	8.20	10.88	6.09	18.64	24.73		
		4	40.2±3.2	2.71±0.91	6.03±3.16	8.73±2.52	6.73±2.33	15.15±8.40	21.88±6.86	8.5±3.2	9.5±3.6
	95		35.7	3.73	0.78	4.51	10.45	2.18	12.63	7.2	9.4
			51.5	5.46	4.35	9.81	10.60	8.45	19.05	5.2	6.1
			37.6	1.80	4.71	6.51	4.79	12.53	17.32	8.9	10.4
			43.6	3.38	6.42	9.80	7.75	14.72	22.47	7.3	lost
		4	42.1±7.1	3.59±1.50	4.07±2.36	7.66±2.61	8.40±2.74	9.47±5.51	17.86±4.10	7.1±1.5	8.6±2.2
10 → 0[4]	95		41.9	2.92	1.31	4.23	6.97	3.13	10.09	<0.01	<0.01
			41.7	2.81	1.55	4.36	6.74	3.72	10.45	<0.01	<0.01
			40.4	2.91	1.25	4.16	7.20	3.09	10.29	<0.01	<0.01
			40.3	2.55	1.73	4.28	6.33	4.29	10.62	<0.01	<0.01
		4	41.1±0.8	2.80±0.17	1.46±0.22	4.26±0.08	6.80±0.37	3.56±0.57	10.36±0.23	<0.01	<0.01

1. The animals used for this experiment were maintained on conventional diet and filter paper bedding.
2. Number of individual observations.
3. Mean ± standard deviation.
4. Placed on a dieldrin-free conventional diet 10 weeks prior to kill.

Table 64. Effect of dieldrin withdrawal on liver DNA concentration and total liver DNA.

Exposure time		Total non-nodular liver DNA[1]		Total nodular liver DNA[1]		Total liver DNA[1] (nodular + non-nodular)		Liver DNA concentration[2,8]	
								non-nodular liver tissue	nodular liver tissue
		68-72 weeks	92-95 weeks	68-72 weeks	92-95 weeks	68-72 weeks	92-95 weeks	92-95 weeks	92-95 weeks
dietary dieldrin[4] ($mg.kg^{-1}$)	n[3]								
0	6						10.7± 0.7[5]	2.59±0.17	
10		15.64	26.74	18.13	5.10	32.77	31.84		
		17.05	13.04	45.85	37.30	62.90	50.34		
		26.77	22.39	19.86	38.30	46.63	60.69		
		24.97	lost[6]	12.15	lost[6]	37.12	lost[6]		
	4	21.1±5.6	20.7±7.0	24.0±14.9	26.9±18.9	45.1±13.0	47.6±14.6	2.76±0.15	2.64±0.33
10 → 0[7]			19.80		8.41		28.21		
			22.18		10.48		32.66		
			23.04		9.28		32.32		
			19.60		12.11		31.71		
			21.1±1.7		10.1± 1.6		31.2± 2.0	3.11±0.19**	2.84±0.13+

1. Expressed in mg.100 g^{-1} bodyweight.
2. Expressed in mg.g^{-1} liver.
3. Number of individual observations.
4. Animals used for this experiment were maintained on conventional diet and filter paper bedding.
5. Mean ± standard deviation.
6. One nodular sample was lost during homogenization.
7. Placed on a dieldrin-free conventional diet 10 weeks prior to kill.
8. Significance of the difference between control (0 mg dieldrin.kg^{-1}) and treatment mean (non-nodular liver tissue): $^{*}p < 0.05$; $^{**}p < 0.01$. Significance of the difference between non-nodular and nodular liver tissue: $^{+}p < 0.05$; $^{++}p < 0.01$.

Table 65. Effect of dieldrin withdrawal on the protein content of liver and subcellular fractions of hepatocytes.

Dietary dieldrin[1] ($mg.kg^{-1}$)	Age (weeks)	n[2]	Nature of the tissue	Total protein[6]		Microsomal protein[6]		Soluble protein[6]	
				$mg.g^{-1}$	$mg.mg^{-1}$ DNA	$mg.g^{-1}$	$mg.mg^{-1}$ DNA	$mg.g^{-1}$	$mg.mg^{-1}$ DNA
0	92	6	non-nodular	202.7±15.7[3]	78.9± 6.4	81.5±5.3	31.5±1.8	65.8±4.4	25.1±1.5
10	95	4	non-nodular	192.1± 6.4	69.9± 5.1*	70.1±4.6**	25.4±1.6***	59.1±3.3*	21.5±2.3*
		3[4]	nodular	196.7± 7.4	76.0±12.5	80.3±5.1	30.7±3.6	62.0±6.0	23.9±5.0
10 → 0[5]	95	4	non-nodular	189.8±10.0	61.2± 4.9**	71.7±6.0*	23.0±0.5***	59.7±3.4*	19.2±1.8
		4	nodular	200.2± 5.2	70.8± 4.8+	69.0±6.2	24.3±1.6	70.2±5.5	24.8±2.3+

1. Animals used for this experiment were maintained on conventional diet and filter paper bedding.
2. Number of individual observations.
3. Mean ± standard deviation.
4. One nodular sample was lost during homogenization.
5. Placed on a dieldrin-free conventional diet 10 weeks prior to kill.
6. Significance of the difference between control (0 mg dieldrin.kg^{-1}) and treatment mean (non-nodular liver tissue): $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. Significance of the difference between non-nodular and nodular liver tissue: $^{+}p < 0.05$; $^{++}p < 0.01$.

Table 66. Effect of dieldrin withdrawal on the specific activity of liver microsomal and soluble enzyme systems.

Dietary dieldrin[1] (mg.kg^{-1})	Age (weeks)	Nature of the tissue	n[2]	*p*-Nitroanisole *O*-Demethylase[3,11]	Epoxide hydratase[4,11]	Glutathione *S*-epoxide transferase[5,11]	UDP-Glucuronyl transferase[6,11]	Glucose-6-phosphatase[7,11]
0	92	non-nodular	6	2.37±0.07[8]	2.11±0.49	0.338±0.034	1.48±0.12	0.268±0.017
10	95	non-nodular	4	8.34±2.11***	5.62±2.40**	0.774±0.150***	2.35±0.25***	0.186±0.061*
		nodular	3[9]	9.87±1.20	7.70±3.05	0.849±0.054++	1.86±0.44	0.077±0.068++
10 → 0[10]	95	non-nodular	4	2.36±0.23	3.67±0.94**	0.442±0.033***	1.83±0.12**	0.262±0.029
		nodular	4	3.81±0.40++	6.51±1.84	0.522±0.126	2.04±0.31	0.102±0.039++

1. Animals used for this experiment were maintained on conventional diet and filter paper bedding.
2. Number of individual observations;
3. Activity is expressed in nmol *p*-nitrophenol formed.mg^{-1} microsomal protein.min^{-1}.
4. Activity is expressed in nmol styrene glycol formed.mg^{-1} microsomal protein.min^{-1}.
5. Activity is expressed in µmol styrene oxide conjugated.mg^{-1} soluble protein.min^{-1}.
6. Activity is expressed in nmol *p*-nitrophenol conjugated.mg^{-1} microsomal protein.min^{-1}.
7. Activity is expressed in µg at P_i.mg^{-1} microsomal protein.min^{-1}.
8. Mean ± standard deviation.
9. One sample was lost during homogenization.
10. Placed on a dieldrin-free conventional diet 10 weeks prior to kill.
11. Significance of the difference between control (0 mg dieldrin.kg^{-1}) and treatment mean (non-nodular liver tissue): *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. Significance of the difference between non-nodular and nodular liver tissue: +$p < 0.05$; ++$p < 0.01$.

Table 67. Effect of dieldrin withdrawal on the activity per unit DNA of liver microsomal and soluble enzyme systems.

Dietary dieldrin[1] ($mg.kg^{-1}$)	Age (weeks)	Nature of the tissue	n[2]	*p*-Nitroanisole *O*-Demethylase[3,11]	Epoxide hydratase[4,11]	Glutathione *S*-epoxide transferase[5,11]	UDP-Glucuronyl transferase[6,11]	Glucose-6-phosphatase[7,11]
0	92	non-nodular	6	74.8± 4.2[8]	68.9±11.9	9.0±1.0	45.8± 2.0	8.86±1.25
10	95	non-nodular	4	210.0±44.0***	142.2±58.9*	16.5±2.8***	58.3± 5.2***	4.72±1.50
		nodular	3[9]	306.2±70.0	231.3±78.2	20.5±5.3	59.9±16.0	2.20±1.66++
10 → 0[10]	95	non-nodular	4	54.5± 4.7***	84.7±21.6	8.5±1.2	42.2± 2.5*	6.03±0.62
		nodular	4	92.4± 9.7++	160.7±54.0+	12.8±2.0	49.7± 8.0	2.48±0.90++

1. Animals used for this experiment were maintained on conventional diet and filter paper bedding.
2. Number of individual observations.
3. Activity is expressed in nmol *p*-nitrophenol formed.mg^{-1} liver DNA.$min.^{-1}$.
4. Activity is expressed in nmol styrene glycol formed.mg^{-1} liver DNA.$min.^{-1}$.
5. Activity is expressed in µmol styrene oxide conjugated.mg^{-1} liver DNA.$min.^{-1}$.
6. Activity is expressed in nmol *p*-nitrophenol conjugated.mg^{-1} liver DNA.$min.^{-1}$.
7. Activity is expressed in µg at P_i.mg^{-1} liver DNA.$min.^{-1}$.
8. Mean ± standard deviation.
9. One nodular sample was lost during homogenization.
10. Placed on a dieldrin-free conventional diet 10 weeks prior to kill.
11. Significance of the difference between control (0 mg dieldrin.kg^{-1}) and treatment mean (non-nodular liver tissue): *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. Significance of the difference between non-nodular and nodular liver tissue: +$p < 0.05$; ++$p < 0.01$.

Table 68. Liver tumour incidence[1].

Diet		SSD				CD			
Bedding		F	S	F	S	F	S	F	S
Dieldrin (mg.kg^{-1} diet)		0	0	10	10	0	0	10	10
Time period (weeks)	*Classification*								
0- 65	Total number of mice	3	2	5	5	8	7	10	3
	No. mice with liver 'A' tumour(s)[2]	0	0	0	1	0	0	2	0
	No. mice with liver 'B' tumour(s)	0	0	0	1	0	0	4	1
	Total liver tumours	0	0	0	2	0	0	6	1
65	Total number of mice	15	15	15	12	15	15	16	16
	No. mice with liver 'A' tumour(s)[2]	2	1	7	10***	0	0	9*	7
	No. mice with liver 'B' tumour(s)	0	0	4	2	0	0	4	5*
	Total liver tumours	2	1	11***	12***	0	0	13***	12***
	% liver tumours	13.3	6.7	73.3	100	-	-	81.3	75.0
66- 90	Total number of mice	10	18[3]	6	2	17	25	21[3]	13
	No. mice with liver 'A' tumour(s)[2]	0	6	1	2**	1	0	10*	4
	No. mice with liver 'B' tumour(s)	0	0	4**	0	0	1	11**	9**
	Total liver tumours	0	6	5**	2**	1	1	21***	13***
	% liver tumours	-	33.3	83.3	100	5.9	4.0	100	100
90- 92	Total number of mice	21	11			21	29		
	No. mice with liver 'A' tumour(s)[2]	0	4*			2	5		
	No. mice with liver 'B' tumour(s)	1	0			0	0		
	Total liver tumours	1	4*			2	5		
	% liver tumours	4.8	36.4			9.5	17.2		
92-110	Total number of mice	6	1	5		7	6	4	6
	No. mice with liver 'A' tumour(s)[2]	0	1	2		1	0	2	2
	No. mice with liver 'B' tumour(s)	0	0	3		0	0	2	4
	Total liver tumours	0	1	5**		1	0	4**	6**
0-110	Total number of mice	55	47	31	19	68	82	51	38
	No. mice with liver 'A' tumour(s)[2]	2	12	10	13	4	5	23	13
	No. mice with liver 'B' tumour(s)	1	0	11	3	0	1	21	19
	Total liver tumours	3	12	21	16	4	6	44	32
	% liver tumours	5.5	25.5	67.7	84.2	5.9	7.3	86.3	84.2

1. Significance of the difference between control (SSD + F + 0 mg dieldrin.kg^{-1}) and treatments: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.
2. Animals which also showed type 'B' liver tumours are not included in the 'A' column.
3. Interim kills were made in this group during this time period.

Table 69. Incidence of type 'B' liver tumours showing lung metastases.

Treatment	Number of mice with type 'B' liver tumours killed or died		Number of mice showing lung metastases		Percentage incidence of lung metastases	
	up to 78 weeks	after 78 weeks	up to 78 weeks	after 78 weeks	up to 78 weeks	after 78 weeks
0 mg dieldrin.kg^{-1} diet	0	2	0	0	0	0
10 mg dieldrin.kg^{-1} diet	27	27	5	13	19	48

References

Agthe, C., H. Cara'a, P. Shubik, L. Tomatis & E. Wenyon, 1970. Study of the potential carcinogenicity of DDT in the Syrian Golden Hamster. Proc. Soc. Exp. Biol. Med. 134: 113.

Alvares, A.P., D.R. Bickers & A. Knappas, 1973. Polychlorinated biphenyls: A new type of inducer of cytochrome P-448 in the liver. Proc. Nat. Acad. Sci. 70: 1321.

Andervont, H.B. & T.B. Dunn, 1948. Efforts to detect a mammary tumour-agent in strain C mice. J. Natl. Cancer Inst. 8: 235.

Andervont, H.B., 1950. Studies on the occurrence of spontaneous hepatomas in mice of strain C3H and CBA. J. Natl. Cancer Inst. 11: 581.

Arcos, J.C., A.H. Conney & N.P. Buu-Hoi, 1961. Induction of microsomal enzyme synthesis by polycyclic aromatic hydrocarbons of different molecular sizes. J. Biol. Chem. 236:1291.

Argyris, T.S. & D. Magnus, 1968. The stimulation of liver growth and demethylase activity following phenobarbital treatment. Dev. Biol. 17: 187.

Argyris, T.S. & D.L. Layman, 1969. Liver growth associated with the induction of demethylase activity after injection of 3-methylcholanthrene in immature rats. Cancer Res. 29:549.

Babish, J.G. & G.S. Stoewsand, 1975. Hepatic microsomal enzyme induction in rats fed varietal cauliflower leaves. J. Nutrition 105: 1592.

Babish, J.G. & G.S. Stoewsand, 1977. Effect of dietary indole-3-carbinol on the induction of the mixed function oxidases of rat tissue. Fd. Cosmet. Toxicol. 16: 151.

Barka, T. & H. Popper, 1967. Liver enlargement and drug toxicity. Medicine 46: 103.

Bartsch, H., C. Malaveille & R. Montesano, 1975. In vitro metabolism and microsome-mediated mutagenicity of dialkylnitrosamines in rat, hamster and mouse tissue. Cancer Res. 35: 644.

Bauer, G., 1978. RNA Tumor-viren. Naturwissensch. Rundschau 11: 445.

Becker, F.F. & B.P. Lane, 1968. Regeneration of the mammalian liver. VI. Retention of phenobarbital-induced cytoplasmic alterations in dividing hepatocytes. Am. J. Path. 52: 211.

Becker, F.F., 1971. Cell function: its importance in chemical carcinogenesis. Fed. Proc. 30: 1736.

Beutler, E., O. Duron & B.M. Kelly, 1963. Improved method for the determination of blood glutathione. J. Lab. Clin. Med. 61: 882.

Bidwell, K., E. Weber, I. Nienhold, T. Connor & M.S. Legator, 1975. Environmental Mutagenic Society, 6th Annual Meeting Program. Pest. Abstracts 8: 676.

Bock, K.W., W. Fröhling, H. Remmer & B. Rexer, 1973. Effects of phenobarbital and 3-methylcholanthrene on substrate specificity of rat liver microsomal UDP-glucuronyltransferase. Biochim. Biophys. Acta (Amst.) 327: 46.

Bock, K.W. & I.N.H. White, 1974. UDP-glucuronyltransferase in perfused rat liver and in microsomes: influence of phenobarbitone and 3-methylcholanthrene. Eur. J. Biochem. 46:451.

Böhm, N. & B. Moser, 1976. Reversibele Hyperplasie und Hypertrophie der Mäuseleber unter funktioneller Belastung mit Phenobarbital. Beitr. Path. 157: 283.

Bolender, R.P. & E.R. Weibel, 1973. A morphometric study of the removal of phenobarbital-induced membranes from hepatocytes after cessation of treatment. J. Cell. Biol. 56: 746.

Botham, C.M., D.M. Conning, J. Hayes, M.H. Litchfield & T.F. McElligott, 1970. Effects of butylated hydroxytoluene on the enzyme activity and ultrastructure of rat hepatocytes. Fed. Cosmet. Toxicol. 8: 1.

Boyland, E. & L.F. Chasseaud, 1969. The role of glutathione and glutathione S-transferases in mercapturic acid biosynthesis. Adv. Enzymol. 32: 173.

Bradley, J.V., 1968. Distribution-free statistical tests (Ed. J.V. Bradley). Prentice Hall, Inc. Englewood Cliffs, New Yersey, p. 195-203.

Burger, P.L. & P.B. Herdson, 1966. Phenobarbital-induced fine structural changes in rat liver. Am. J. Pathol. 48: 793.

Burton, K., 1956. A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem. 62: 315.
Butler, W.H., 1978. The effects of long-term administration of phenobarbitone to rats. Brit. J. Cancer 37: 418.
Chasseaud, L.F., 1973. The nature and distribution of enzymes catalysing the conjugation of glutathione with foreign compounds. Drug. Metal. Rev. 2: 185.
Chasseaud, L.F., 1976. Conjugation with glutathione and mercapturic acid excretion. In: Glutathione, Metabolism and Function, (Eds. I.M. Arias & W.B. Jakoby). Raven Press, New York, p. 77-114.
Clemmesen, J., V. Fuglsang-Frederiksen & C.M. Plum, 1974. Are anti-convulsants oncogenic? Lancet 1: 705.
Clemmesen, J., 1975. Phenobarbitone, liver tumours and thorotrast. Lancet 1: 37.
Conney, A.H., C. Davison, R. Gastel & J.J. Burns, 1960. Adaptive increases in drug-metabolizing enzymes induced by phenobarbital and other drugs. J. Pharmacol. Exp. Ther. 130: 1.
Conney, A.H., 1967. Pharmacological implications of microsomal enzyme induction. Pharmacol. Rev. 19: 317.
Craddock, V.M., 1971. Liver carcinomas induced in rats by single administration of dimethylnitrosamine after partial hepatectomy. J. Natl. Cancer Inst. 47: 899.
Craddock, V.M., 1975. Effect of a single treatment with the alkylating carcinogens dimethylnitrosamine, diethylnitrosamine and methyl methane sulphate on liver regenerating after partial hepatectomy. I. Test for induction of liver carcinomas. Chem. Biol. Interact. 10: 313.
Crampton, R.F., T.J.B. Gray, P. Grasso & D.V. Parke, 1977. Long-term studies on chemically induced liver enlargement in the rat. I. Sustained induction of microsomal enzymes with absence of liver damage on feeding phenobarbitone or butylated hydroxytoluene. Toxicology 7: 289.
Crowley, C., B. Gillham & M.B. Thorn, 1975. A direct enzymic method for the determination of reduced glutathione in blood and other tissues. Biochem. Med. 13: 287.
Czygan, P., H. Greim, J.A. Garro, F. Utterer, E. Schaffner, M. Popper & O. Rosenthal, 1973. Microsomal metabolism of dimethylnitrosamine and the cytochrome P-450 dependency of its activation to a mutagen. Cancer Res. 33: 2983.
Davis, L.J. & O.G. Fitzhugh, 1962. Tumourigenic potential of aldrin and dieldrin for mice. Toxicol. Appl. Pharmacol. 4: 184.
Darby, F.J. & R.K. Grundy, 1975. Glutathione S-aryltransferase: the effect of treating male and female rats with phenobarbitone on the apparent kinitic parameters for the conjugation of 1,2-dichloro-4-nitrobenzene and 1-chloro-2,4-dinitrobenzene with glutathione. Biochem. J. 128: 175.
Dean, B.J. & S.M.A. Doak, 1975. The potential mutagenicity of dieldrin (HEOD) in mammals. Food Cosmet. Toxicol. 13: 317.
Deichmann, W.B., M. Keplinger, F. Sala & E. Glass, 1967. Synergism among oral carcinogens. IV. The simultaneous feeding of four tumourigens to rats. Toxicology Appl. Pharmacol. 11: 88.
Della Porta, G. & B. Terracini, 1969. Chemical carcinogenesis in infant animals. Progr. exp. Tumour Res. 11: 334.
Depierre, J.W. & L. Ernster, 1976. Disappearance of induced endoplasmic reticulum after cessation of phenobarbital treatment. FEBS Letters 68: 219.
Deringer, M.K., 1965. Occurrence of mammary tumours, reticular neoplasms and pulmonary tumours in strain BALB/c Ar. De breeding female mice. J. Natl. Cancer Inst. 35: 1047.
Dietary standards for laboratory rats and mice, 1969. Recommendations of the Laboratory Animals Association Nutrition Study Group, Laboratory Handbook 2, Laboratory Animals Ltd., London.
Doljanski, F., Z. Eshkol, D. Givol, E. Kaufmann & E. Margoliash, 1956. The effect of large doses of thiourea on the composition of the liver and urine of rats. J. Endocrinol. 13: 141.
Dutton, G.J., 1966. The biosynthesis of glucuronides. In: Glucuronic acid, Free and Combined, Ed. G.J. Dutton. Academic Press, New York, p. 185-299.
Epstein, C.J., H.L. Moses, L.B. Epstein & M.M. Garrison, 1967. A structural analysis of hepatomegaly induced by a hormone-secreting tumour. Exp. Mol. Pathol. 7: 304.
Estabrook, R.W., M.R. Franklin, B. Cohen, A. Shigamatzu & A.G. Hildebrandt, 1971. Influence of hepatic microsomal mixed function oxidation reactions on cellular metabolic control. Metabolism 20: 187.
Ferguson, H.C., 1966. The effect of red chedar chip bedding on hexobarbital and pentobarbital sleeping time. J. Pharm. Sci. 55: 1142.

Ferrigan, L.W., C.G. Hunter & D.E. Stevenson, 1965. Observation on the effects of continued oral exposure of rats to dieldrin. Fd. Cosmet. Toxicol. 3: 149.
Feuer, G., L. Golberg & J.R. Le Pelley, 1965. Liver response tests. I. Exploratory studies on glucose-6-phosphatase and other liver enzymes. Food Cosm. Toxicol. 3: 235.
Fiala, S., A. Mohindru, W.G. Kettering, A.E. Fiala & H.P. Morris, 1976. Glutathione and gamma glutamyl transpeptidase in rat liver during chemical carcinogenesis. J. Nat. Cancer Inst. 57: 591.
Fiske, C.H. & P. Subbarow, 1925. The colorimetric determination of phosphorus. J. Biol. Chem. 66: 375.
Fitzhugh, O.G. & A.A. Nelson, 1947. The chronic oral toxicity of DDT (2,2-bis(p-chlorophenyl-1,1,1-trichloroethane)). J. Pharmacol. 89: 18.
Fitzhugh, O.G., A.A. Nelson & J.P. Frawley, 1950. The chronic toxicities of technical benzene hexachloride and its alpha, beta and gamma isomers. J. Pharmacol. Exp. Therap. 100: 59.
Fitzhugh, O.G., A.A. Nelson & M.L. Quaife, 1964. Chronic oral toxicity of aldrin and dieldrin in rats and dogs. Food Cosmet. Toxicol. 2: 551.
Fouts, J.R. & L.A. Rogers, 1965. Morphological changes in the liver accompanying stimulation of microsomal drug metabolizing enzyme activity by phenobarbital, chlordane, benzpyrene or methylcholanthrene in rats. J. Pharmacol. Exp. Ther. 147: 112.
Gilbert, D.& L. Golberg, 1965. Liver response tests. III. Liver enlargement and stimulation of microsomal processing enzyme activity. Food Cosmet. Toxicol. 3: 417.
Gilbert, D. & L. Golberg, 1967. BHT oxidase. A liver microsomal enzyme induced by the treatment of rats with butylated hydroxyltoluene. Food Cosmet. Toxicol. 5: 481.
Gilbert, D., A.D. Martin, S.D. Gangolli, R. Abraham & L. Golberg, 1969. The effects of substituted phenols on liver weights and liver enzymes in the rat: structure-activity relationships. Food Cosmet. Toxicol. 7: 603.
Gillette, J.R., D.C. Davis & H.A. Sasame, 1972. Cytochrome P-450 and its role in drug metabolism. Ann. Rev. Pharmacol. 12: 57.
Graillot, C., J.C. Gak, C. Lancret & R. Truhaut, 1975. Recherches sur les modalités et les mecanismes d'action toxique des insecticides organochlorés. II. Etude chez le Hamster des effects de toxicité a long terme du DDT. Eur. J. Toxicol. 8: 353.
Grasso, P. & R.F. Crampton, 1972. The value of the mouse in carcinogenicity testing. Food Cosmet. Toxicol. 10: 418.
Grasso, P. & J. Hardy, 1974. Strain difference in natural incidence and response to carcinogens. In: Mouse Hepatic Neoplasia, Eds. W.H. Butler and P.M. Newberne. Elsevier, Amsterdam/New York, p. 111-129.
Grisham, J.W., 1973. Effects of drugs on hepatic cell proliferation. In: Drugs and the Cell Cycle. Academic Press, New York/London, p. 95.
Gross, L., 1954. Is leukemia caused by a transmissible virus? A working hypothesis. Blood, 9: 557.
Gross, L., 1974. The role of viruses in the etiology of cancer and leukemia. J. Am. Med. Assoc. 230: 1029.
Gross, L., 1978. Viral etiology of cancer and leukemia: a look into the past, present and future- G.H.A. Clowes memorial lecture. Cancer Res. 38: 485.
Harper, A.E., 1965. Glucose-6-phosphatase. In: Methods of Enzymatic Analysis. Ed. H.H. Bergmeyer. Academic Press, New York/London, p. 788-792.
Haugen, D.A. & M.J. Coon, 1976. Properties of electrophoretically homogeneous phenobarbital inducible and β-naphthoflavone-inducible forms of liver microsomal cytochrome P-450. J. Biol. Chem. 251: 7929.
Haugen, D.A., M.J. Coon & D.W. Nebert, 1976. Induction of multiple forms of mouse liver cytochrome P-450. Evidence for genetically controlled de novo protein synthesis in response to treatment with β-naphthoflavone or phenobarbital. J. Biol. Chem. 251: 1817.
Hoch-Ligeti, G., M.F. Argus & S.C. Arcos, 1968. Combined carcinogenic effects of dimethylnitrosamine and 3-methylcholanthrene in the rat. J. Natl. Cancer Inst. 40: 535.
Hodge, H.C., A.M. Boyce, W.B. Deichmann & H.F. Kraybill, 1967. Toxicology and no-effect levels of aldrin and dieldrin. Toxicol. Appl. Pharmacol. 10: 613.
Hötzel, D. & R.H. Barnes, 1966. Contributions of the intestinal microflora to the nutrition of the host. Vitams. Horm. 24: 115.
Hoffmann, D.G., H.M. Worth, J.L. Emmerson & R.C. Anderson, 1970. Stimulation of hepatic drug-metabolizing enzymes by chlorophenothane (DDT): the relationship to liver enlargement and hepatotoxicity in the rat. Toxicol. Appl. Pharmacol. 16: 171.
Hoogendam, I., J.P.J. Versteeg & M. de Vlieger, 1962. Electroencephalograms in insecticide toxicity. Arch. Environ. Health 4: 86.
Hoogendam, I., J.P.J. Versteeg & M. de Vlieger, 1965. Nine years toxicity control in insecticide plants. Arch. Environ. Health 10: 441.

Hunter, C.G. & J. Robinson, 1967. Pharmacodynamics of dieldrin (HEOD): I. Ingestion by human subjects for 18 months. Arch. Environm. Health 15: 614.
Hunter, C.G., J. Robinson & M. Roberts, 1969. Pharmacodynamics of dieldrin (HEOD). Ingestion by human subjects for 18 to 24 months and post-exposure for 8 months. Arch. Environm. Health 18: 12.
Hutson, D.H., 1976. The comparative metabolism of dieldrin in the rat (CFE) and in two strains of mouse (CF-1 and LACG). Food Cosmet. Toxicol. 14: 577.
Ito, N.,H. Nagasaki, M. Arai, S. Sugihara & S. Makiura, 1973. Histologic and ultrastructural studies on the hepatocarcinogenicity of benzene hexachloride in mice. J. Natl. Cancer Inst. 51: 817.
Ito, N., H. Nagasaki, H. Aoe, S. Sugihara, Y. Miyata, M. Arai, & T. Shirai, 1975. Development of hepatocellular carcinomas in rats treated with benzene hexachloride. J. Natl. Cancer Inst. 54: 801.
Jager, K.W., 1970. Aldrin, Dieldrin, Endrin and Telodrin - An Epidemiological and Toxicological Study of Long-term Occupational Exposure. Elsevier, Amsterdam/London/New York.
Jakoby, W.B., W.H. Habig, J.H. Keen, J.N. Ketley & M.J. Pabst, 1976. Glutathione S-transferases: catalytic aspects. In: Glutathione, Metabolism and Function. Eds. I.M. Arias and W.B. Jakoby. Raven Press, New York, p. 189-211.
James, M.O., J.R. Fouts & J.R. Bend, 1976. Hepatic and extrahepatic metabolism, in vitro, of an epoxide (8-^{14}C-styrene oxide) in the rabbit. Biochem. Pharmacol. 25: 187.
Jelnick, B., M.C. Katayama & A.E. Harper, 1952. The inadequacy of unmodified potato starch as dietary carbohydrate for the albino rat. Can. J. Med. Sci. 30: 447.
Jenna, D.M. & J.R. Bend, 1977. Glutathione S-transferases. In: Biological reactive intermediates: Formation, Toxicity and Inactivation. Eds. D.J. Jollow, J.J. Kocsis, R. Suyder & H. Vainio. Plenum Press, New York. p. 207-236.
Johnson, M.K., 1966. Metabolism of iodomethane in the rat. Biochem. J. 98: 38.
Jones, G. & W.H. Butler, 1975. Morphology of spontaneous and induced neoplasia. In: Mouse Hepatic Neoplasia. Eds. W.H. Butler & P.M. Newberne. Elsevier, Amsterdam/Oxford/ New York, p. 21-60.
Kaplowitz, N., J. Kuhlenkamp & G. Clifton, 1975. Drug induction of hepatic glutathione S-transferases in male and female rats. Biochem. J. 146: 351.
Kiely, J.M., J.L. Titus & A. Orvis, 1973. Thorotrast-induced hepatoma presenting as hyper parathyroidism. Cancer 31: 1312.
Kimura, N.T., T. Kanematsu & T. Baba, 1976. Polychlorinated biphenyl(s) as a promotor in experimental hepatocarcinogenesis in rats. Z. Krebsforsch. 87: 257.
Kitigawa, T., 1971. Histochemical analysis of hyperplastic lesions and hepatomas of the liver of rats fed 2-fluorenylacetamide. Gann 62: 217.
Kitigawa, T. & H. Sugano, 1977. Enhancement of azo-dye hepatocarcinogenesis with dietary phenobarbital in rats. Gann 68: 255.
Klassen, C.D. & G.L. Plaa, 1968. Studies on the mechanism of phenobarbital-enhanced sulphobromophtalein disappearance. J. Pharmacol. Exp. Ther. 161: 361.
Klatskin, G., 1977. Hepatic tumours: possible relationship to use of oral contraceptives. Gastroenterology 73: 386.
Koransky, W., S. Magour, H.J. Merker, I. Schlicht & R. Schulte-Hermann, 1966. Influence of inducing substances on growth of liver and microsomal electron transport systems. Proceedings Third International Pharmacological Meeting, Vol. 4. Pergamon Press, Elmsford, New York, p. 55.
Koransky, W., S. Magour, G. Nosck & R. Schulte-Hermann, 1969. Ueber den Einfluss induzierender Substanzen auf Fremdstoff-Oxydasen und andere Redoxenzyme der Leber. Naunyn-Schmiedebergs Arch. Pharmak. Exp. Pathol. 263: 281.
Kunz, W., G. Schaude, W. Smidt & M. Siess, 1966a. Lebervergrösserung durch Fremdstoffe. Naunyn-Schmiedebergs. Arch. Pharmak. Exp. Path. 254: 470.
Kunz, W., G. Schaude, W. Schmidt & M. Siess, 1966b. Stimulation of liver growth by drugs. I. Morphological analysis. Proc. Eur. Soc. for Study of Drug Tox. 7: 113.
Kunz, W., G. Schaude & M. Siess, 1967. Die Beeinflussung der Nitrosamincarcinogenese durch Leberwachstum induzierende Fremdstoffe. Abstracta European Cancer Meeting, Vienna. Vienna Academy Publishers, p. 47.
Kunz, W., G. Schaude & C. Thomas, 1969. Die Beeinflussung der Nitrosamincarcinogenese durch Phenobarbital und Halogenkohlenwasserstoffe. Z. Krebsforsch. 72: 291.
Kunz, W. & B. Schnieders, 1970. RNA metabolism and induction of extramicrosomal enzymes during liver enlargement due to drugs. Proc. 4th. Internat. Congr. Pharmacol. 4: 326.
Kunz, W., K.E. Appel, R. Rickart, M. Schwarz & G. Stöckle, 1978. Enhancement and inhibition of carcinogenic effectiveness of nitrosamines. In: Primary Livers Tumours. Eds. H. Remmer, H.M. Bolt, P. Bannasch & H. Popper. MTP Press Ltd., Lancaster, U.K., p. 261-284.

Lane, B.P. & C.S. Lieber, 1967. Effects of butylated hydroxytoluene on the ultrastructure of rat hepatocytes. Lab. Invest. 16: 342.
Laws, E.R., A. Curley & F.J. Biros, 1967. Men with intensive occurpational exposure to DDT: A clinical and chemical study. Arch. Environm. Health 15: 766.
Laws, E.R., A. Curley & F.J. Biros, 1967. Men with intensive occupational exposure to to DDT: effect on the human liver. Arch. Environm. Health 27: 318.
Lehman, A.J., 1965. DDT (a mixture of 1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane and 1,1,1-trichloro-2-(O-chlorophenyl)-2-(p-chlorophenyl) ethane). In: Summaries of pesticide toxicity, Food and Drug Administration, U.S. Department of Health, Education and Welfare, Washington DC, U.S., p. 17.
Loub, W.D., L.W. Wattenberg & D.W. Davis, 1975. Aryl hydrocarbon hydroxylase induction in rat tissues by naturally occurring indoles of cruciferous plants. J. Natl. Cancer Inst. 54: 985.
Lowry, O.H., N.J. Rosebrough, A.L. Farr & R.J. Randall, 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265.
Lu, A.Y.H., A. Somogyi, S. West, R. Kunteman & A.H. Conney, 1972. Pregnelonone-16α-carbonitrile: A new type of inducer if drug-metabolizing enzymes. Arch. Biochem. Biophys. 152: 457.
Lu, A.Y.H., A. Somogyi, S. West, R. Kunteman & A.H. Conney, 1972. Pregnelenone-16α-carbonitrile: A new type of inducer of drug-metabolizing enzymes. Arch. Biochem. Biophys. 152: 457.
Lu, A.Y.H. & W. Levin, 1974. The resolution and reconstitution of the liver microsomal hydroxylation system. Biochem. Biophys. Acta 344: 205.
Lapis, K., 1978. Pathogenesis and biological features of the virus-induced primary liver cancer in chickens and its established transplantable form. In: Primary Liver Tumours, Eds. H. Remmer, H.M. Bolt, P. Bannasch & H. Popper. MTP Press Ltd., Lancaster, U.K., p. 437-448.
MacMahon, H.E., A.S. Murphy & M.I. Bates, 1947. Endothelial-cell sarcoma of liver following thorotrast injections. Amer. J. Path. 23: 585.
Madison, R.M., L.S. Rabstein & W. Ray Bryan, 1968. Mortality rate and spontaneous lesions found in 2,928 untreated BALB/c Cr mice. J. Natl. Cancer Inst. 40: 683.
Makiuara, S., H. Aoe, S. Sugihara, K. Hirao, M. Arai & N. Ito, 1974. Inhibitory effect of polychlorinated biphenyls on liver tumourigenesis in rats treated with 3-methyl-4-dimethylaminoazobenzene, N-2-fluorenylacetamide and diethylnitrosamine. J. Natl. Cancer Inst. 53: 1253.
Mann, N.S., A. Chaudry, S. Thaler & A Sachder, 1976. Hepatoma induced by thorium dioxide. South Med. J. 69: 510.
Meldolesi, J., 1967. On the significance of the hypertrophy of the smooth endoplasmic reticulum in liver cells after administration of drugs. Biochem. Pharmacol. 16: 125.
Milkovic, S., M.M. Garrison & R.W. Bates, 1964. Study of the hormonal control of body and organ size in rats with mammotrophic tumours. Endocrinology 75: 670.
Miller, J.A., 1970. Carcinogenesis by chemicals: An overview. Cancer Res. 30: 559.
Moron, M.S., J.W. DePierre, K. Jacobsson & B. Mannervik, 1977. Levels of glutathione and glutathione-metabolizing enzymes in rat lung. In: Microsomes and Drug Oxidations. Eds. Ullrich, Roots, Hildebrandt, Estabrook & Conney). Pergamon Press, Oxford, p. 447-452.
Nagasaki, H., S. Tomii, T. Mega, M. Marugami & N. Ito, 1971. Development of hepatomas in mice treated with benzenehexachloride. Gann 62: 431.
Nagasaki, H., S. Tomii, T. Mega, M. Marugami & N. Ito, 1972. Carcinogenicity of benzenehexachloride (BHC). In: Topics in Chemical Carcinogenesis. Eds. W. Nakahara, S. Takayama, T. Sigimura & S. Odashima. Tokyo, University of Tokyo Press, p. 343.
National Cancer Institute, 1978a. Carcinogenesis Technical Report Series, No. 21. Bioassays of aldrin and dieldrin for possible carcinogenicity. U.S. Department of Health Education and Welfare.
National Cancer Institute, 1978b. Carcinogenesis Technical Report Series, No. 22. Bioassay of dieldrin for possible carcinogenicity. U.S. Department of Health, Education and Welfare. Public Health Service, National Institutes of Health
Netter, K.J. & G. Seidel, 1964. An adaptively stimulated O-demethylating system in rat liver microsomes and its kinetic properties. J. Pharm. Exptl. Ther. 146: 61.
Nishizumi, M., 1976. Enhancement of diethylnitrosamine hepatocarcinogenesis in rats by exposure to polychlorinated biphenyls or phenobarbital. Cancer Letters 2: 11.
Oesch, F., D.M. Jerina & J. Daly, 1971. A radiometric assay for epoxide hydrase activity with $|7\text{-}^{3}H|$-styrene oxide. Biochim. Biophys. Acta 227: 685.
Oesch, F., N. Morris, J.W. Daly, J.E. Gielen & D.W. Nebert, 1973. Genetic expression of the induction of epoxide hydrase and arylhydrocarbonhydroxylase activities in the mouse by phenobarbital or 3-methyl-cholanthrene. Molec. Pharmacol. 9: 692.

Oesch, F., 1975. Biochemistry of the mammalian systems involved in biosynthesis and inactivation of carcinogenic and potentially carcinogenic epoxides. Chimia 29: 66.
Ohde, G., J. Schuppler, R. Schulte-Hermann & H. Keiger, 1979. Proliferation of rat liver cells in pre-neoplastic nodules after stimulation of liver growth by xenobiotic inducers. Acrh. Toxicol. Suppl. 2: 451.
Orlowski, M. & A. Meister, 1970. The γ-glutamyl cycle: a possible transfer system for amino acids. Proc. Natl. Acad. Sci. USA 57: 1248.
Ortega, P., W.J. Hayes & W.E. Durham, 1957. Pathologic changes in the liver of rats after feeding low levels of various insecticides. Arch. Pathol. 64: 614.
Ortega, P., 1966. Light and electronmicroscopy of dichlorodiphenyl-trichloroethane (DDT) poisoning in rat liver. Lab. Invest. 15: 657.
Ortelee, M.F., 1958. Study of men with prolonged intensive occupational exposure to DDT. Arch. Indust. Health 18: 433.
Owen, N.V., W.J. Griffing, D.G. Hoffman, W.R. Gibson & R.C. Anderson, 1971. Effects of dietary administration of 5-(3,4-dichlorophenyl)-5-ethylbarbituric acid (dichlorophenobarbital) to rats. Emphasis on hepatic drug-metabolizing enzymes and morphology. Toxicol. Appl. Pharmacol. 18: 720.
Pascal, G., G. Durand & E. Penot, 1970. Influence de l'ingestion de di-tertiobutylhydroxytoluène (BHT) sur la croissance corporelle et sur la composition du tissu hepatique du rat blanc. Arch. Sci. Physiol. 24: 37.
Peraino, C., R.J.M. Fry & E. Staffeldt, 1971. Reduction and enhancement by phenobarbital of hepatocarcinogenesis induced in the rat by 2-acetylaminofluorene. Cancer Res. 31: 1506.
Peraino, R.J., R.J.M. Fry & E. Staffeldt, 1973a. Enhancement of spontaneous hepatic tumourigenesis in C_3H mice by dietary phenobarbital. J. Natl. Cancer Inst. 51: 1349.
Peraino, C., R.J.M. Fry, E. Staffeldt & W.E. Kisieleski, 1973b. Effects of varying the exposure to phenobarbital on its enhancement of 2-acetylaminofluorene-induced hepatic tumourigenesis in the rat. Cancer Res. 33: 2701.
Peraino, C., R.J.M. Fry, E. Staffeldt & J.P. Christopher, 1975. Comparative enhancing effects of phenobarbital, amobarbital, diphenylhydantoin and dichlorodiphenyltrichloroethane on 2-acetylaminofluorene induced hepatic tumourigenesis in the rat. Cancer Res. 35: 2884.
Peraino, C., R.J.M. Fry, E. Staffeldt & J.P. Christopher, 1977. Enhancing effects of phenobarbitone and butylated hydroxytoluene on 2-acetylaminofluorene-induced hepatic tumourigenesis in the rat. Food Cosm. Toxicol. 15: 93.
Pogell, B.M. & C.R. Krisman, 1960. Enzymic hydrolysis of uridine-diphosphateglucuronate in rat skin. Bioch. Bioph. Acta 41: 349.
Ponomarkov, V. & L. Tomatis, 1976. The effect of long-term administration of phenobarbitone in CF-1 mice. Cancer Letters 1: 165.
Pound, A.W. & T.A. Lawson, 1975. Partial hepatectomy and toxicity of dimethylnitrosamine and carbon tetrachloride, in relation to the carcinogenic action of dimethylnitrosamine. Brit. J. Cancer 32: 596.
Preis, C., G. Schaude & M. Siess, 1966. Histometrische Analysen der Lebervergrösserung nach chronischer Einwirkung von Barbituraten und Halothan. Naunyn-Schmiedebergs Arch. Pharmak. Exp. Pathol. 254: 489.
Raalte, H.G.S. van, 1973. Of hepatomas mice and man. In: Pesticides and the environment: a continuing controversy, Eight Inter-American Conference on Toxicology and Occupational Medicine. Ed. W.B. Deichmann. Intercontinental Medical Book Corporation, New York, London, p. 245-252.
Radomski, J.L., W.B. Deichmann, W.E. MacDonald & E.M. Glass, 1965. Synergism among oral carcinogens. I. Results of simultaneous feeding of four tumourigens to rats. Toxicol. Appl. Pharmacol. 7: 652.
Raisfeld, J.H., P. Baccin, F. Hutterer & F. Schaffner, 1970. The effect of 3-amino-1,2,4-triazole on the phenobarbital-induced formation of hepatic microsomal membranes. Mol. Pharmacol. 6: 231.
Remmer, H. & H.J. Merker, 1963. Enzyminduktion und Vermehrung von endoplasmatischem Reticulum in der Leberzelle während der Behandlung mit Phenobarbital (Luminal). Klin. Wochensch. 41: 276.
Rossi, L., M. Ravera, G. Repetti & L. Santi, 1977. Long-term administration of DDT or phenobarbital-Na in Wistar rats. Int. J. Cancer 19: 179.
Scherer, E., M. Hoffman, P. Emmelot & M. Friedrich-Freksa, 1972. Quantitative study on foci of altered liver cells induced in the rat by a single dose of diethylnitrosamine and partial hepatectomy. J. Natl. Cancer Inst. 49: 93.
Scherer, E. & P. Emmelot, 1975. Foci altered liver cells induced by a single dose of diethylnitrosamine and partial hepatectomy: their contribution to hepatocarcinogenesis in the rat. Europ. J. Cancer 11: 145.

Scherer, E. & P. Emmelot, 1976. Kinetics of induction and growth of enzyme-deficient islands involved in hepatocarcinogenesis. Cancer Res. 36: 2544.
Schlicht, I., W. Koransky, S. Magour & R. Schulte-Hermann, 1968. Grösse und DNS-synthese der Leber unter dem Einfluss körperfremder Stoffe. Naunyn-Schmiedebergs Arch. Pharmak. Exp. Pathol. 261: 26.
Schneidermann, M.A., 1974. Phenobarbitone and liver tumours. Lancet II: 1085.
Schoental, R., 1974. Role of podophyllotoxin in the bedding and dietary zearalenone on the incidence of spontaneous tumours in laboratory animals. Cancer Res. 34: 2419.
Schulte-Hermann, R., W. Koransky, C. Leberl & G. Noack, 1971. Hyperplasia and hypertrophy of rat liver induced by α-hexachlorocyclohexane and butylhydroxytoluene. Retention of the hyperplasia during involution of the enlarged organ. Virchows Arch. Abt. B. Zellpath. 9: 125.
Schulte-Hermann, R., 1974a. Induction of liver growth by xenobiotic compounds and other stimuli. Crit. Rev. Toxicol., 3: 97.
Schulte-Hermann, R., C. Leberl, H. Landgraf & W. Koransky, 1974b. Liver growth and mixed function oxidase activity, dose-dependent stimulatory and inhibitory effects of α-hexachlorocyclohexane. Naunyn-Schmiedebergs Arch. Pharmacol. 285: 355.
Schulte-Hermann, R., 1977. Stimulation of liver growth and mixed function oxidase by α-hexachlorocyclohexane: separation of inductive pathways. In: Microsomes and Drug Oxidations. Eds. V. Ullrich et al. Pergamon Press, Oxford/New York, p. 559-568.
Schulte-Hermann, R., 1978. Induction of liver growth by drugs and tumour promotion. In: Primary Liver Tumours. Eds. H. Remmer, H.M. Bolt, P. Bannasch & H. Popper. MTP Press, Lancaster, p. 385-394.
Schulte-Hermann, R., 1979. Reactions of the liver to injury: adaptation. In: Toxic Injury of the Liver. Eds. E. Farber and M.M. Fisher. Marcel Dekker, New York (in press).
Sherlock, S., 1978. Hepatic tumours and sex hormones. In: Primary Liver Tumours. Eds. H. Remmer, H.M. Bolt, P. Bannasch & H. Popper. MTP Press Ltd., Lancaster, U.K., p. 201-212.
Smith, C.S. & H.I. Pilgrim, 1971. Spontaneous neoplasms in germ-free BALB/cPi mice. Proc. Soc. Exp. Biol. (N.Y.) 138: 542.
Smoron, G.L., & H.A. Battifora, 1972. Thorotrast-induced hepatoma. Cancer 30: 1252.
Springfield, A.C., G.P. Carlson & I.I. De Feo, 1973. Liver enlargement and modification of hepatic microsomal drug metabolism in rats by pyethrum. Toxicol. Appl. Pharmacol. 24: 298.
Stäubli, W., R. Hess & E. Weibel, 1969. Correlated morphometric and biochemical studies on the liver cell. II. Effects of phenobarbital on rat hepatocytes. J. Cell. Biol. 42: 92.
Stier, A., W. Kunz, A.K. Walli & H. Schimassek, 1972. Effects on growth and metabolism of rat liver by halothane and its metabolite trifluoroacetate. Biochem. Pharmacol. 21: 2181.
Stonard, M.D. & J.B. Greig, 1976. Different patterns of hepatic microsomal enzyme activity produced by administration of pure hexachlorobiphenyl isomers and hexachlorobenzene. Chem. Biol. Interact. 15: 365.
Terracini, B., M.C. Testa, J.R. Cabral & N. Day., 1973a. The effects of long-term feeding of DDT to BALB/C mice. Int. J. Cancer 11: 747.
Terracini, B., R.J. Cabral & M.C. Testa, 1973b. A multigeneration study on the effects of continuous administration of DDT to BALB/C mice. In: Proceedings of the 8th Inter-American Conference on Toxicology: Pesticides and the environment, a continuing controversy. Ed. W.B. Deichmann. Miami, Florida, 1973. New York, London, Intercontinental Medical Book Corporation, p. 77-85.
Thorpe, E. & A.I.T. Walker, 1973. The toxicology of dieldrin (HEOD). II. Comparative long-term oral toxicity studies in mice with dieldrin, DDT, phenobarbitone, α-BHC and β-BHC. Food Cosm. Toxicol. 11: 433.
Thorpe, E., 1973. The toxicology of dieldrin (HEOD); Transplantation of liver tumours in mice. Tunstall Laboratory Group Research Report, TLGR. 0042.73.
Thorpe, E. & P.F. Hunt, 1975. Toxicology of dieldrin (HEOD): Study of the pathological changes in three strains of mice following prolonged ingestion of dieldrin. Tunstall Laboratory Group Research Report, TLGR. 0012.75.
Thomas, P.E., A.Y.H. Lu, D. Ryan, S.B. West, J. Kawalek & W. Levin, 1976. Immunochemical evidence for six forms of rat liver cytochrome P-450 obtained using antibodies against purified rat liver cytochromes P-450 and P-448. Molec. Pharmacol. 12: 746.
Tomatis, L., V. Turosov, N. Day & R.T. Charles, 1972. The effects of long-term exposure to DDT on CF-1-mice. Int. J. Cancer 10: 489.
Tomatis, L., C. Partensky & R. Montesano, 1973. The predictive value of mouse liver tumour induction in carcinogenicity testing. Int. J. Cancer: 12, 1.

Tomatis, L., V. Turosov, R.T. Charles, M. Boiocchi & E. Gati, 1974. Liver tumours in CF-1 mice exposed for limited periods to technical DDT. Z. Krebsforsch. 82: 25.
Turosov, V.S., N.E. Day, L. Tomatis, E. Gati & R.T. Charles, 1973. Tumours in CF-1 mice exposed for six consecutive generations to DDT. J. Natl. Cancer Inst. 51: 983.
Vaino, H. & M.G. Parkki, 1976. Enhancement of microsomal mono-oxygenase, epoxide hydratase and UDP-glucuronyl transferase by aldrin, dieldrin and isosafrole administrations in rat liver. Toxicology 5: 279.
Versteeg, J.P.J. & K.W. Jager, 1973. Long-term occupational exposure to the insecticides aldrin, dieldrin, endrin and telodrin. Brit. J. Ind. Med. 30: 201.
Vessell, E.S., 1967. Induction of drug-metabolizing enzymes in liver microsomes of mice and rats by softwood bedding. Science 157: 1057.
Villeneuve, J.P., P. Mavier & J.G. Joly, 1976. Ethanol-induced cytochrome P-450: catalytic activity after partial purification. Biochem. Biophys. Res. Commun. 70: 723.
Walker, A.I.T., D.E. Stevenson, J. Robinson, E. Thorpe & M. Roberts, 1969. The toxicology of dieldrin (HEOD). Two years oral exposures of rats and dogs. Toxicol. Appl. Pharmacol. 15: 345.
Walker, A.I.T., E. Thorpe & D.E. Stevenson, 1973. The toxicology of dieldrin (HEOD). I. Long-term oral toxicity studies in mice. Food Cosm. Toxicol. 11: 415.
Weisburger, J.H. & E.K. Weisburger, 1968. Food additives and chemical carcinogens: on the concept of zero tolerance. Food Cosmet. Toxicol. 6: 235.
Weisburger, J.H., R.M. Madison, J.M. Ward, Ch. Viguera & E.K. Weisburger, 1975. Modification of diethylnitrosamine liver carcinogenesis with phenobarbital but not with immunosuppression. J. Natl. Cancer Inst. 54: 1185.
Wright, A.S., D. Potter, M.F. Wooder, C. Donninger & R.D. Greenland, 1972. The effects of dieldrin on subcellular structure and function of mammalian liver cells. Food Cosmet. Toxicol. 10: 311.
Wright, A.S., D.A.A. Akintonwa, & M.F. Wooder, 1977. Studies on the interactions of dieldrin with mammalian liver cells at the subcellular level. Ecotoxicol. and Environ. Safety 1: 7.
Wright, A.S., C. Donninger, R.D. Greenland, K.L. Stemmer & M.R. Zavon, 1978. The effects of prolonged ingestion of dieldrin on the livers of male rhesus monkeys. Ecotoxicol. Environm. Safety 1: 477.