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[*Contents continued*]

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INFORMATION SECTION

ARTICLES OF GENERAL INTEREST*

TOXICOLOGY: BIBRA ABSTRACTS AND COMMENTS*

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Research Section

DETERMINATION OF BENZO[*a*]PYRENE IN SMOKED, COOKED AND TOASTED FOOD PRODUCTS

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(*Received 29 January 1979*)

Abstract—Samples of smoked, cooked and toasted food products, commercially available in Italy, and some samples of oils and fats were analysed for benzo[*a*]pyrene. In the commercial samples, benzo[*a*]pyrene was detected in approximately 73% of the samples, at levels ranging from 0·01 to 9·51 μg/kg. In electrically-broiled foods, the benzo[*a*]pyrene content ranged from 0 to 0·05 μg/kg. Levels of benzo[*a*]pyrene found in the food products analysed were lower than levels reported by other investigators for comparable food commodities.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are experimentally well established carcinogens, and there is a wealth of information concerning the effects of these chemicals on various tissues and organs of laboratory animals (IARC, 1973). In man, exposure has not been to individual PAHs, but to combinations as they occur in soot, coal-tar, pitch and mineral oils as well as in other substances including tobacco smoke, exhaust, smoked and charcoal-broiled foods.

In this investigation attention has been focused on benzo[*a*]pyrene both for historical reasons, since it was the first hydrocarbon to be identified and recognized as a carcinogen (Rhee & Bratzler, 1970), and because it is widely considered as a general indicator for the presence of other carcinogens in the group.

Cooked meat, smoked food, charcoal-broiled meat and vegetable oils are some common foods in which PAHs have been detected and quantified (Fretheim, 1976; Haenni & Fischbach, 1974; Howard & Fazio, 1969; Lo & Sandi, 1978).

Several analytical methods, which are applicable to a number of PAHs, have been developed, and are available for the detection and quantitative determination of PAHs in environmental and biological samples (Howard, Turicchi, White & Fazio, 1966; van Langermeersch, 1968). Ultra-violet absorption and fluorescence spectrophotometry are often employed (IARC, 1971); however, gas chromatography combined with mass spectrometry (GC–MS) is now the favoured method, particularly when detailed analysis is required (Schaad, 1970). High pressure liquid chromatography (HPLC) has been explored and it has found useful application for the separation, identification and determination of various PAHs (Nakagawa, Sato, Kawamura, Watabe & Morita, 1978; O'Hara, Chin, Dainius & Kilbuck, 1974; Sleight, 1973).

This paper reports the results obtained using GC–MS for the determination of benzo[*a*]pyrene in smoked, cooked, toasted food products and some oils and fats commercially available in Italy. Literature searches demonstrate that extensive monitoring for benzo[*a*]pyrene in foods has been undertaken and that, in some cases, legal limits have been suggested and established for this food contaminant (Toth, 1971). In Italy, analytical data are sporadic and fragmentary; this investigation was initiated to contribute to the development of more systematic data in this domain and the present communication gives the results so far of monitoring studies which are still in progress.

EXPERIMENTAL

Chemicals. All solvents used in these studies (methanol, cyclohexane and *N*,*N*-dimethylformamide) were purified by distillation. For adsorption chromatography, the silica gel employed (kieselgel 60, 70–230 mesh ASTM, Merck) was activated for 12 hr at 150°C and then deactivated by adding 15% (w/v) distilled water. Benzo[*a*]pyrene (Fluka, Switzerland) was used as the external standard.

Methods. Polycyclic aromatic hydrocarbons were extracted following the technique of Grimmer & Böhnke (1975) with the following modifications: after chromatography on silica gel (gel treated as described above), the sample was concentrated almost to dryness under a stream of nitrogen, diluted to a known volume with cyclohexane, and then submitted to selected ion monitoring. For the quantitative determination of benzo[*a*]pyrene, mass fragmentography was used according to the method proposed by van Cauwenberghe & Cautreels (1976). The analyses were carried out on an LKB9000S GC–MS system using a glass column 2 m × 3 mm, packed with 3% OV-101 on Chromosorb W-HP, 80/100 mesh. The GC–MS operating conditions were as follows: oven temperature (isothermal): 250°C; temperatures of the injection port and molecular separator: 280°C and 250°C, respectively; carrier gas: helium, flow rate: 30 ml/min.

Selected ion monitoring was used with a 20 eV ionization energy and the ion focalized at $m/e = 252$. The quantitative analysis was performed using an external standard. Benzo[*a*]pyrene was the standard, and care was taken to exclude contamination of the gas chromatography apparatus by injecting pure solvent after each standard or sample application. The percentage recovery of added benzo[*a*]pyrene ranged from 93 to 95%. It should be noted that the GC column employed for the selected ion monitoring did not permit the separation of the two isomeric forms benzo[*a*]- and benzo[*e*]pyrene; however contemporaneous analyses of some samples on a capillary column demonstrated that the content of benzo[*e*]-pyrene was negligible.

RESULTS AND DISCUSSION

The levels of benzo[*a*]pyrene in smoked food products are reported in Table 1. The same table also reports values found in the literature for comparable types of foodstuffs. The samples analysed showed amounts of benzo[*a*]pyrene lower than those found by other investigators; only in the case of *provola* (buffalo milk cheese), did the benzo[*a*]pyrene content exceed 1 μg/kg, and even then it was not nearly so high as the values reported by Mannelli (1966) for the same food.

Table 2 reports the benzo[*a*]pyrene content of samples of raw and cooked meat. *Porchetta* (baked sucking pig) is a traditional foodstuff in the central part of Italy; it is home-prepared by roasting the whole piglet on an open wood fire. It is apparent from the results, that the largest amount of benzo[*a*]-pyrene is found in the outside of the roasted piglet. The raw and grilled hamburgers were prepared from the same quality ground meat used for commercial purposes and it was purposely chosen for its relatively high fat content. Hamburgers were cooked on an electric grill specially designed, having a water-filled pan underneath the electric resistance. In this particular type of grill water creates an air vortex which prevents the dripping from falling on the incandescent coil, thus avoiding the formation of smoke and pyrolysis. The results obtained appear to give some support to the efficiency of this type of grill since no difference in content of benzo[*a*]pyrene was observed between the grilled meat and sausage and the raw.

Data obtained from the analysis of toasted food products are reported in Table 3. This table also reports values reflecting the effect of toasting-time on samples of bread: toasting periods of 3 and 5 min determined relative increments of benzo[*a*]pyrene of 70 and 144%, respectively. These values suggest that it would be advisable to pay attention to the toasting time to prevent the formation of high levels of benzo[*a*]pyrene on toast. The same table reports amounts of benzo[*a*]pyrene found in tea and coffee, as powder and leaves as well as infusions: while the benzo[*a*]pyrene content of coffee powder appears to

Table 1. *Benzo*[a]*pyrene content of smoked food products*

Product	Benzo[*a*]pyrene (μg/kg)	
	Observed values*	Literature values
Wurstel	0·46	0·4–2·9 (Fritz, 1968a; Lucisano, De Battistis & Marzadori, 1973; Mannelli, 1966)
Smoked *provola*†	1·28	4·10–6·20 (Mannelli, 1966)
Smoked bacon	0·25	0·2–4·6 (Fritz, 1968b; Mannelli, 1966; Rhee & Bratzler, 1970)
Fried smoked bacon	0·16	0·4–0·7 (Rhee & Bratzler, 1970)

* Values represent the means of at least two determinations.
† Buffalo milk cheese.

Table 2. *Benzo*[a]*pyrene content of cooked food products*

Product	Benzo[*a*]pyrene (μg/kg)	
	Observed values*	Literature values
Fat ground meat (hamburger):		
raw	0·05	—
cooked†	0·05	2·6–20·0 (Lijinski & Ross, 1967; Panalaks, 1976)
Cooked sausage†	0·05	—
Baked sucking pig:		
rind	1·22	—
flesh	0·79	—

*Values represent the means of at least two determinations.
†Cooked on an electric grill especially designed to prevent smoke formation and pyrolysis.

Table 3. *Benzo*[a]*pyrene content of toasted food products*

Product	Benzo[a]pyrene (μg/kg)	
	Observed value*	Literature value
Bread	0·23	0·2–0·3 (Fritz, 1968c)
Toasted bread (3 min)	0·39	—
Toasted bread (5 min)	0·56	0·5 (Fritz, 1968d)
Coffee (powder)	0·80	0·3–0·5 (Fritz, 1968c; IARC, 1973; Panalaks, 1976)
Coffee (infusion)	0·01	0·0–4·0 (Kuratsune & Hueper, 1960; Maier & Stender, 1969)
Tea (leaves)	9·51	0·5–21·3 (Howard & Fazio, 1969; IARC, 1973)
Tea (infusion)	0·02	—

*Values represent the means of at least two determinations.

Table 4. *Benzo*[a]*pyrene content of edible fats and oils*

Product	Benzo[a]pyrene (μg/kg)	
	Observed value*	Literature value
Olive oil:		
raw	3·59	0·4–0·5 (Howard & Fazio, 1969; Howard, Turicchi, White & Fazio, 1966)
fried	2·36	0·9–1·9 (Howard & Fazio, 1969; Malanoski, Greenfield, Barnes, Worthington & Joe, 1968)
Butter	0·47–0·91	—

*Values represent the means of at least two determinations.

be high and that of tea leaves substantially higher, the content of the infusions was very low. This might be worth noting since infusions represent the commonest methods of tea and coffee preparation.

Data from the analysis of fats and oils are found in Table 4. The olive oil examined was *Olio Extra Vergine di Oliva*® (extra pure olive oil, Registered Brand). The results obtained from the oil are in agreement with the values reported in the literature for edible oils, even though most of the available data are from seed oils. With regard to the values on the reduction in benzo[a]pyrene content in samples of fried oil, the results are comparable to those reported by Fritz (1968b) which were not recorded in the table; the reduction may be due to the formation of peroxidases during heating which could possibly decompose the hydrocarbons present in the oil. The same observation applies to the values obtained from the fried smoked bacon reported in Table 1. The benzo[a]pyrene values obtained from butter, however, do not find confirmation in the literature since Fabian (1969) reported the presence of benzo[a]pyrene in coconut oil and margarine but not in butter.

Table 5. *Benzo*[a]*pyrene content of some bovine tissues*

Tissues	Bovine	Age	Benzo[a]pyrene (μg/kg)*
Muscle (lean)	A	8	0·15
Muscle (lean)	B	9	0·10
Muscle (lean)	C	11	0·27
Adipose	A	8	0·09
Adipose	B	9	0·07
Adipose	C	11	0·25
Perirenal fat	A	8	0·13
Perirenal fat	B	9	0·14
Perirenal fat	C	11	0·03

*Values represent the mean of at least two determinations.

Table 5 gives the benzo[a]pyrene contents of adipose and muscle tissues from three adult bovines (8–11 yr of age), traditionally fed that is with the exclusion of any synthetic or integrated feed. Owing to the lack of information in the literature, it is not possible to draw any conclusions about the significance of these results.

REFERENCES

Fabian, B. (1968). Carcinogenic substances in edible fats and oils. Part IV: Studies on margarine, vegetable fats and butter (in German). *Arch. Hyg.* **152** (3), 231.

Fretheim, K. (1976). Carcinogenic polycyclic aromatic hydrocarbons in Norwegian smoked meat sausages. *J. agric. Fd Chem.* **24**, 976.

Fritz, W. (1968a). Scientific Report. Benzo[*a*]pyrene and other polycyclic aromatic hydrocarbons in margarine and mayonnaise (in German). *Nahrung* **12** (4), 495.

Fritz, W. (1968b). On the formation of carcinogenic hydrocarbons during the thermal treatment of foods. Part IV: The effect of frying (in German). *Nahrung* **18** (8), 809.

Fritz, W. (1968c). On the formation of carcinogenic hydrocarbons during the thermal treatment of foods. Part II: The roasting of coffee-beans and coffee-substitutes (in German). *Nahrung* **152** (3), 231.

Fritz, W. (1968d). On the formation of carcinogenic hydrocarbons during the thermal treatment of foods. Part III: The baking of bread and biscuits (in German). *Nahrung* **12** (8), 805.

Grimmer, G. & Bohnke, H. (1975). Polycyclic aromatic hydrocarbons profile analysis of high-protein foods, oils and fats by GLC. *J. Ass. off. analyt. Chem.* **58**, 725.

Haenni, E. O. & Fischbach, H. (1974). Trace polynuclear aromatic hydrocarbons analysis. In *The Contribution of Chemistry to Food Supplies. IUPAC-IUFOST Symposium.* p. 209. Butterworths, London.

Howard, J. W. & Fazio, T. (1969). A review of polycyclic aromatic hydrocarbons in food. *J. agric. Fd Chem.* **17**, 527.

Howard, J. W., Turicchi, E. W., White, R. H. & Fazio, T. (1966). Food additives—Extraction and estimation of polycyclic aromatic hydrocarbons in vegetable oils. *J. Ass. off. analyt. Chem.* **49**, 1236.

IARC (International Agency for Research on Cancer) (1971). Standardization of Sampling and Analytical Procedures for Estimating Polynuclear Hydrocarbons in the Environment. *IARC Int. Tech. Rep. Ser. No.* 71/002.

IARC (International Agency for Research on Cancer) (1973). *IARC Monograph on the Evaluation of Carcinogenic Risk of Chemicals to Man. Vol. 3. Certain polycyclic aromatic hydrocarbons and heterocyclic compounds.* IARC, Lyon. p. 91.

Kuratsune, M. & Hueper, W. C. (1960). Polycyclic aromatic hydrocarbons in roasted coffee. *J. natn. Cancer Inst.* **24**, 463.

Lijinski, W. & Rose, A. E. (1967). Production of carcinogenic polynuclear hydrocarbons in the cooking of food. *Fd Cosmet. Toxicol.* **5**, 343.

Lo, M. T. & Sandi, E. (1978). Polycyclic aromatic hydrocarbons (polynuclears) in foods. *Res. Rev.* **69**, 35.

Lucisano, A., De Battistis, P. & Marzadori (1973). Investigation on 3,4-benzopyrene in smoked foods (in Italian). *Vet. Ital.* **24**, 232.

Maier, H. G. & Stender, W. (1969). Carcinogenic hydrocarbons in coffee-substitutes (in German). *Deut. Lebensm.-Runds.* **11**, 341.

Malanoski, A. J., Greenfield, E. L., Barnes, C. J., Worthington, J. M. & Joe, F. L., Jr. (1968). Food additives—Survey of polycyclic aromatic hydrocarbons in smoked foods. *J. Ass. off. analyt. Chem.* **51**, 114.

Mannelli, G. (1966). Presence of benzopyrene in smoked foods (in Italian). *Ann. Fac. Econ. Comm.* **4** (2), 469.

Nakagawa, T., Sato, Y., Kawamura, T., Watabe, A. & Morita, M. (1978). Determination of benzo[*a*]pyrene in liquid paraffin by HPLC. *Bull. env. contam & Toxicol. (U.S.).* **19** (6), 703.

O'Hara, J. R., Chin, M. S., Dainius, B. & Kilbuck J. H. (1974). Determination of benzo[*a*]pyrene in smoke condensates by high pressure rapid liquid-liquid chromatography. *J. Fd Sci.* **39**, 38.

Panalaks, T. (1976). Determination and identification of PAHs in smoked and charcoal broiled food products by HPLC and GLC. *J. Envir. Sci. Hlth.* **4**, 299.

Rhee, K. S. & Bratzler, L. J. (1970). Benzo[*a*]pyrene in smoked meat products. *J. Fd Sci.* **35**, 146.

Schaad, R. E. (1970). Chromatography of carcinogenic polycyclic aromatic hydrocarbons (in German). *Chromat. Rev.* **13**, 61.

Sleight, R. B. (1973). Reversed phase chromatography of some aromatic hydrocarbons. Structure-retention relationship. *J. Chromat.* **83**, 31.

Toth, L. (1971). Polycyclic hydrocarbons in smoked ham and bacon (in German). *Fleischwirtschaft* **51**, 1069.

van Cauwenberghe, K. & Cautreels, W. (1976). Determination of polyaromatic hydrocarbons in airborne particular matter by mass fragmentography. In *Advances in Mass Spectrometry in Biochemistry and Medicine. Vol. 1.* p. 465. Spectrum Publications, Inc., London.

van Langermeersch, A. (1968). Identification and quantitation of carcinogenic hydrocarbons (in French). *Chimie analyt.* **50**, 3.

Fd Cosmet. Toxicol. Vol. 17, pp. 329 to 331

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THE MUTAGENICITY OF SOY BEAN SAUCE

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(*Received 19 July 1978*)

Abstract—Soy bean sauce is commonly used as a food material in Taiwan where the incidence of hepatoma and gastric cancer is high. We have therefore investigated the mutagenicity of soy bean sauce *in vitro*. When treated with nitrite at the 2000 ppm level soy bean sauce produced a mutagenic substance as demonstrated using the *Salmonella*/mammalian microsome mutagenicity test. All 21 different brands of soy bean sauce showed similar results. Most mutagenic material was formed when the nitrite level was 2000 ppm and the pH was 3; ascorbic acid prevented the formation of mutagenic products in nitrite-treated soy bean sauce.

INTRODUCTION

Carcinomas of the liver and stomach are the most common malignant tumours among Chinese people living in Taiwan, and cancer has been the second commonest cause of death since 1964 (Lin, Chang & Chen, 1977; Yeh, 1966).

In a search for the aetiological factors contributing to these malignant tumours, the short-term carcinogenicity testing method developed by Ames (Ames, McCann & Yamasaki, 1975) was used to examine the extracts of food materials particularly favoured by Chinese people in the preparation of food. The samples were treated with or without sodium nitrite at pH 3 and five strains TA98, TA100, TA1535, TA 1537 and TA1538 of histidine-requiring auxotrophs of *Salmonella typhimurium* kindly supplied by B. N. Ames were used for these experiments.

EXPERIMENTAL

Materials. *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine and 2-aminofluorene were obtained from Tokyo Kasei Kogyo Co. (Tokyo, Japan). Picrolonic acid was purchased from Sigma Chemical Co. (St. Louis, MO, USA). NADP and glucose 6-phosphate were obtained from Calbiochem (San Diego, CA, USA). The food materials were purchased from the local stores in Taipei City.

Extraction of samples. About 600 ml of each soy bean sauce sample were first concentrated under reducing pressure with a flush evaporator to 200 ml. Except for the dose-response experiment and ascorbic-acid treated samples, 2000 ppm $NaNO_2$ was added to 300 ml aliquots of soy bean sauce. Nothing was added to the control samples. The reaction was carried out for 1 hr at 25°C and pH 3, and it was terminated by the addition of 2000 ppm ammonium sulphamate. The reaction mixtures were cooled to 4°C and extracted twice with 200 ml ether. The combined ether extracts were reduced under a stream of nitrogen to 0·5 ml, and then diluted with 5·5 ml of dimethylsulphoxide (DMSO) for assaying.

Mutagenicity assay. The mutagenicity assay was carried out using *S. typhimurium* according to the method of Ames *et al.* (1975). S-9 fraction, 9000 **g** liver supernatant, from Aroclor-treated male albino rats (500 mg/kg), was used for metabolic activation of the mutagens possibly present in the samples (Ames *et al.* 1975). The samples were incubated at 37°C for 48 hr, and then the results were recorded. *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine (2 μg/plate), and picrolonic acid (250 μg/plate) were used as positive controls with TA100, 1535 and with TA98, 1537 and 1538 respectively. Neither of these compounds requires metabolic activation, 2-aminofluorene (250 μg/plate) was used as a positive control with the S-9 fraction for TA1538. Each assay was performed twice quite independently, and the data presented are the average of two experiments.

In order to determine the optimal pH for producing the highest numbers of revertant colonies, the samples were treated with 2000 ppm sodium nitrite at pH 1, 2, 3 and 4. To determine the optimum nitrite level, nitrite levels of 1000, 2000, 3000, 4000, 5000 and 10,000 ppm were added to the 300-ml samples of soy bean sauce. The quantity of the final DMSO extract of the nitrite-treated soy bean sauce was also varied to determine whether there was a dose-response. The effect of adding 13,000 ppm ascorbic acid was also determined using the reaction mixture containing 5000 ppm of sodium nitrite at pH 3.

RESULTS

For the indicator organisms, TA1535 and 100, in the presence of S-9 fraction, the optimum pH was found to be 3 (Fig. 1). A positive dose-response relationship was found between the concentration of sodium nitrite and the number of revertant colonies for the indicator organisms *S. typhimurium* TA1535 and 100 in the presence of S-9 fraction (Figs 2a and b). Nitrite alone does not cause an increase in the number of revertant colonies at concentrations of up to 500 μg/plate.

The increase in the number of revertant colonies was proportional to the amounts of DMSO extract applied, using tester strains TA100 and 1535 (Figs 3a and b). Control samples of soy bean sauce without

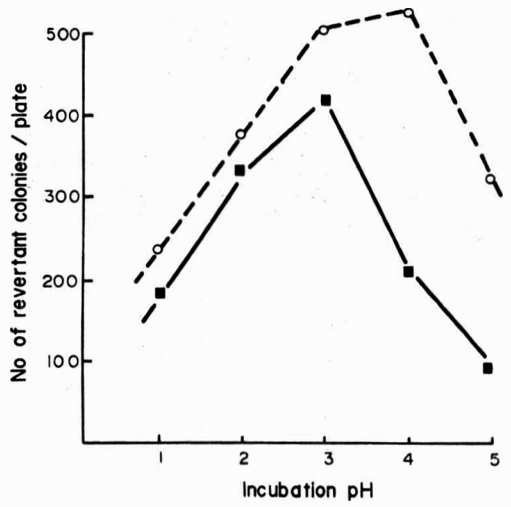

Fig. 1. The mutagenic response of *S. typhimurium*, strains TA100 (—○—) and 1535 (—■—) in the presence of S-9 fraction, to soy bean sauce (sample code name OP) treated with 2000 ppm nitrite, at various pHs.

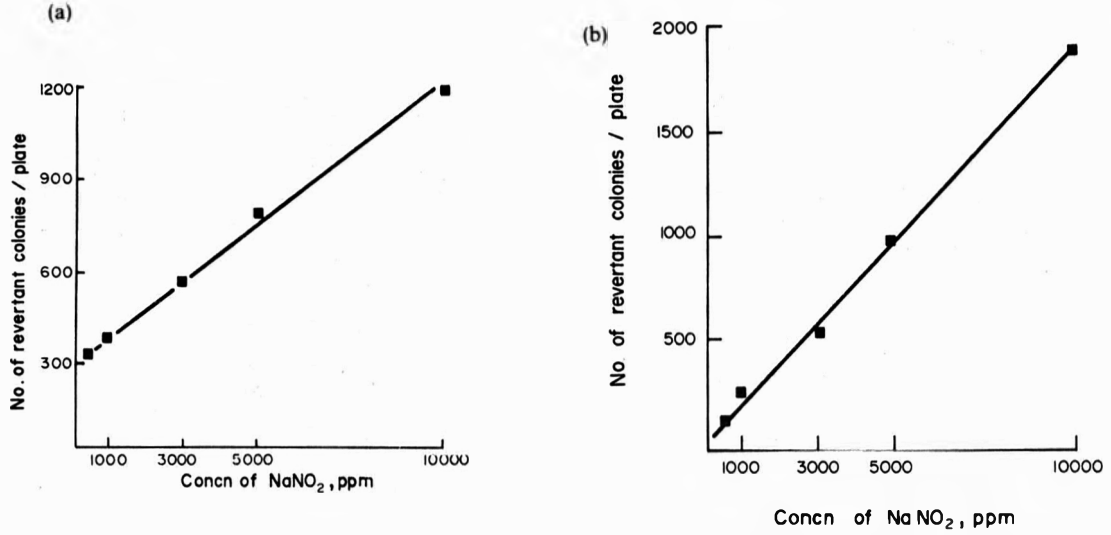

Fig. 2. The mutagenic response of *S. typhimurium* strains TA100(a) and 1535(b) in the presence of S-9 fraction, to soy bean sauce (sample code name OP) treated with various concentrations of nitrite.

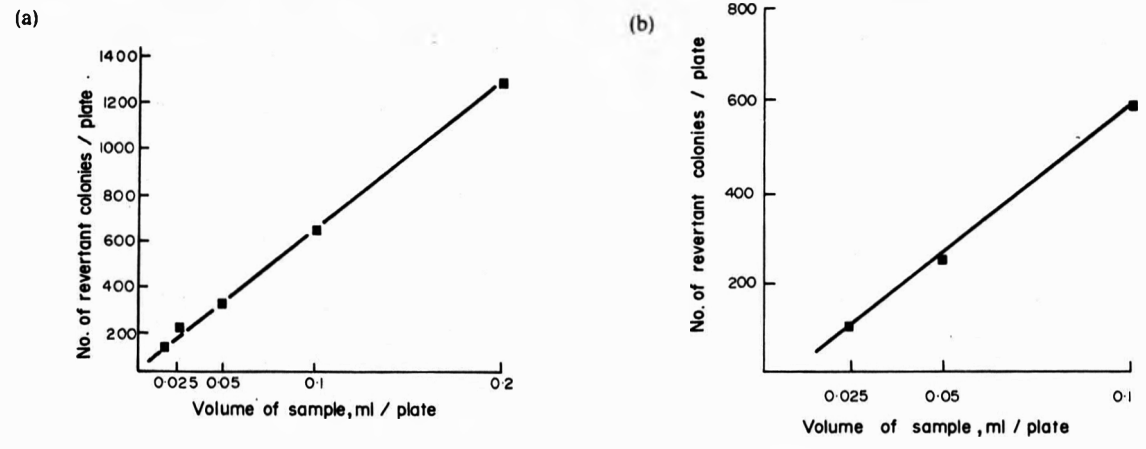

Fig. 3. The mutagenic response of *S. typhimurium* strains TA100(a) and 1535(b) in the presence of S-9 fraction, to various amounts of soy bean sauce (sample code name OP) pretreated with 2000 ppm nitrite.

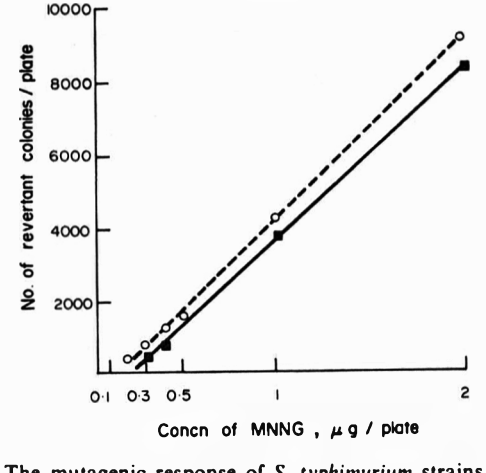

Fig. 4. The mutagenic response of *S. typhimurium* strains TA100 (—○—) and 1535 (—■—) to various amounts of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG).

nitrite treatment had no effect on strains TA100 and 1535.

Incubation with ascorbic acid, prevented the formation of the mutagenic materials as tested by using strains *S. typhimurium* TA100 and 1535.

The results of dose-response experiments with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine using TA1535 and 100 are shown in Fig. 4. Picrolonic acid (250 μg/plate) produced an average of 1700, 430 and 1265 revertant colonies per plate with TA98, 1537 and 1538 respectively. 2-Aminofluorene (10 μg/plate) produced 234 revertant colonies with TA1538 in the presence of S-9.

DISCUSSION

All 21 different brand names of soy bean sauce, brought from the local stores of Taipei City, contain an unknown substance which reacts to form a compound of the alkylnitrosoureido type by nitrosation *in vitro*. The mutagenic activity of the resulting compound was revealed using strains of *S. typhimurium* (TA1535 and 100) that are susceptible to mutation through base-pair substitution. Although less revertants were obtained if the S-9 fraction was not added during the analysis, there was very little difference in the number of revertants produced by the extracts of soy bean sauce in the presence or absence of S-9 fraction after the mutagen(s) were purified by thin-layer chromatography.

Soy bean sauce is a very popular flavour for use in Chinese cooking. The 16 million people on this island annually consume 14 to 15 million dozen bottles (each bottle containing 600 ml) of soy bean sauce. Chinese people in Taiwan are currently using nitrite in the food industry for several purposes, including protection against botulism in meat and fish products (Chen & Lin, 1977; Lin, 1978).

Nitrosamines and nitrosamides were first reported to be toxic and carcinogenic by Barnes & Magee (1954), and these compounds may play a part in the occurrence of cancer in man since it was shown that nitrosamines can be formed from their precursors in animal organs (Sander, 1971), and that feeding of amine and nitrite together produced tumours in animals (Druckrey, 1975; Greenblatt, Mirvish & So, 1971; Lijinsky & Taylor, 1977; Magee & Barnes, 1954; Sugimura & Kawachi, 1973).

Nitrosable products have been detected in fish (Marquardt, Rufino & Weisburger, 1977; Neurath, Dünger, Pein, Ambrosius & Schreiber, 1977), which was suspected of being an aetiological factor in the high incidence of gastric cancer in Japan.

Comparing the mutagenic effects of nitrite-treated raw fish with soy bean sauce, 0·8 ml of soy bean sauce is equivalent to 1 g of raw fish in its power to induce the reversion of *S. typhimurium* TA1535 and 100 based on the data presented by Marquardt, Rufino & Weisburger in 1977.

It was also found that some of the nitrite-treated sauce samples also contained mutagen(s) that caused the reversion of the tester strains, such as *S. typhimurium* TA1538.

These mutagens could originate from the materials used in the preparation of soy bean sauce or from metabolic products produced by fungi or bacteria during fermentation. These two possibilities and the nature of nitrosable compounds are currently being investigated. The animal experiments to examine whether the mutagens found in nitrosated soy bean sauce are also carcinogens are being carried out.

Acknowledgement—This work was supported in part by a grant from the National Council of Science, Republic of China.

REFERENCES

Ames, B. N., McCann, J. & Yamasaki, E. (1975). Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test. *Mutation Res.* **31**, 347.

Barnes, J. M. & Magee, P. N. (1954). Some toxic properties of dimethylnitrosamine. *Br. J. ind. Med.* **11**, 167.

Chen, L. C. & Lin, C. Y. (1977). Studies on adapted additive and substitute of nitrite in cured meat products (in Chinese). *National Science Council Monthly* (*R.O.C.*) **5**, 107.

Druckrey, H. (1975). Chemical carcinogenesis of *N*-nitroso derivatives *Gann Monogr.* **17**, 107.

Greenblatt, M., Mirvish, S. & So, B. T. (1971). Nitrosamine studies: Induction of lung adenomas by concurrent administration of sodium nitrite and secondary amines in Swiss mice. *J. natn. Cancer Inst.* **46**, 1029.

Lijinsky, W. & Taylor, H. W. (1977). Feeding tests in rats on mixtures of nitrite with secondary and tertiary amines of environmental importance. *Fd Cosmet. Toxicol.* **15**, 269.

Lin, J. K. (1978). Studies on the levels and toxicities of nitrite and nitrosamine in Chinese Food (in Chinese). *National Science Council Monthly* (*R.O.C.*) **6**, 155.

Lin, T. M., Chang, L. C. & Chen, K. P. (1977). A statistic analysis on mortality of malignant neoplasms in Taiwan. *J. Formosan Med. Ass.* **76**, 656.

McCann, J., Choi, E., Yamasaki, E. & Ames, B. N. (1975). Detection of carcinogens as mutagens in the *Salmonella*/mammalian microsome test: assay of 300 chemicals. *Proc. natn. Acad. Sci. U.S.A.* **72**, 5135.

Magee, P. N. & Barnes, J. M. (1967). Carcinogenic nitroso compounds. *Adv. Cancer Res.* **10**, 163.

Marquardt, H., Rufino, F. & Weisburger, J. H. (1977). On the aetiology of gastric cancer: mutagenicity of food extracts after incubation with nitrite. *Fd Cosmet. Toxicol.* **15**, 97.

Neurath, G. B., Dünger, M., Pein, F. G., Ambrosius, D. & Schreiber, O. (1977). Primary and secondary amines in the human environment. *Fd Cosmet. Toxicol.* **15**, 275.

Sander, J. (1971). Untersuchungen über die Entstehung cancerogener Nitrosoverbindungen im Magen von Versuchstieren und ihre Bedeutung für den Menschen. 1-3 Mitteilung. *Arzneimittel-Forsch.* **21**, 1572, 1707 & 2034.

Sugimura, T. & Kawaachi, T. (1973). Experimental Stomach Cancer. In *Methods in Cancer Research*. Edited by H. Bush. p. 245. Academic Press. Inc., New York.

Yeh, S. (1966). Some geographic aspects of most common diseases in Taiwan. Part II. Infection and cancer. *Int. Path.* **7**, 81.

Fd Cosmet. Toxicol. Vol. 17. pp. 333 to 338

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STUDIES ON THE INTERACTION OF METHOMYL AND ETHANOL IN RATS

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Abstract—The effects of feeding methomyl (200 mg/kg diet) and ethanol (10% aqueous solution) both separately and together were studied in R/Amsterdam rats. Response to the combined administration of the two agents produced evidence of some interactions, resulting in a decrease in growth in both male and female rats and an increase in the relative weights of the adrenals. Sex differences were apparent, however, in some of the responses to the combined treatment: only in the male rats were hepatic levels of triglyceride and free fatty acids increased and the cholinesterase activity of the brain decreased to a level significantly below those associated with administration of either agent separately, whereas in the females the relative weights of the kidneys and the fasting level of blood-glucose were increased.

INTRODUCTION

Methomyl (1-methylthioacetaldehyde-*O*-(methylcarbamoyl)oxime; Lannate®) is a broad-spectrum insecticide. In Hungary, the granting of approval for the widespread application of methomyl is now under consideration.

According to the *Information Bulletin* issued by the Du Pont Company, the oral LD_{50} is 17–24 mg/kg for starved male or female rats and 37 mg/kg for fed rats. Studies on labelled methomyl have indicated rapid metabolism and no accumulation of the insecticide, which is eliminated from the organism in 24 hr as carbon dioxide, acetonitrile and an unidentified urinary metabolite. A potential acute hazard is the inhibition of cholinesterase.

In an earlier study in this laboratory, the LD_{50} determined for methomyl in rats of our own stock was the same as that reported by the Du Pont Company. In a subacute feeding study in rats, the no-effect level for methomyl has been calculated as 100 mg/kg diet, a value also in agreement with corresponding data from Du Pont. On feeding a dietary level of 200 mg methomyl/kg, serum levels of total lipid and cholesterol were raised in the rats. The purpose of the work now described was to study the combined effects of methomyl and a second toxic agent, ethanol, on growth and some metabolic parameters of rats.

EXPERIMENTAL

Chemicals. Methomyl (1-methylthioacetaldehyde-*O*-(methylcarbamoyl)oxime; *S*-methyl-*N*-[(methylcarbamoyl)oxy]thioacetimidate), with a purity of 90%, was a generous gift from E. I. Du Pont de Nemours and Co. Inc., Industrial and Biochemicals Department, Wilmington, DE, USA. The substance was a white crystalline powder with a mild odour of sulphur, melting at 78–79°C, and readily soluble in water and organic solvents. Ethanol (96%) was glass-distilled before use.

Animals and diet. Lean R/Amsterdam albino rats, which are not prone to gain weight, were housed in wire cages. Room temperature was 20 ± 1°C with a relative humidity of 50–60%. The animals were fed a semi-synthetic diet containing (per kg): casein 180 g, sucrose 368 g, wheat starch 368 g, sunflower oil 40 g, salt mixture 34 g and vitamin mixture 10 g. The 34 g of salt mixture consisted of $CaHPO_4.2H_2O$ 25·76 g, $KHCO_3$ 3·417 g, KH_2PO_4 1·625 g, NaCl 1·27 g, $MgCO_3$ 1·387 g, $CuSO_4.5H_2O$ 19·6 mg, KI 0·196 mg, $C_6H_5FeO_7.3H_2O$ 133·8 mg, $MnCl_2$ 114·5 mg and $ZnCl_2$ 25 mg. The vitamin mixture comprised choline chloride 2000 mg, *m*-inositol 300 mg, DL-calcium pantothenate 20 mg, *p*-aminobenzoic acid 5 mg, nicotinic acid 20 mg, biotin 0·10 mg, folic acid 1·5 mg, thiamine.HCl 4 mg, pyridoxine.HCl 3 mg, riboflavin 6 mg, α-tocopherol 120 mg, menadione 30 mg, retinol 12 mg and cholecalciferol 1000 IU, made up to 10 g by the addition of glucose.

Experimental design and conduct. R/Amsterdam albino rats were divided into four groups of ten males (weighing 175–220 g) and ten females (110–150 g). The control group received the semi-synthetic diet and tap-water, a second group was fed basal diet to which 200 mg methomyl/kg had been added, the third was given untreated diet and drinking fluid containing 10% ethanol, while the fourth received the 200 mg methomyl/kg diet and 10% ethanol as drinking-fluid. The semi-synthetic diet was made semi-solid by addition of fluid (0·5–0·6 ml/g), tap-water for the first two groups and 10% aqueous ethanol for the other two.

Food and fluid intakes were recorded daily and the rats were weighed fortnightly. At week 12, haematocrit and fasting blood-glucose levels were determined, using the Galenopharm Gluco GOD/POD/PAP enzymatic test method, and at the end of week 12 the animals were killed by decapitation and the liver, kidneys, adrenals, heart and spleen were weighed and studied by light microscopy. After rapid removal

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and weighing of the liver, samples were dissected out, weighed and processed for biochemical studies. Brain tissue was also removed for study.

Studies of liver biochemistry. Liver glycogen was estimated by the enzymatic (Galenopharm) determination of glucose formed following hydrolysis with sulphuric acid (Good, Kramer & Somogyi, 1933). Liver samples were extracted by the method of Folch, Lees & Sloane-Stanley (1957) for the estimation of triglycerides (Varsányi Nagy, 1974) and free fatty acids (Mosinger, 1965). For the determination of glucose-6-phosphate dehydrogenase (G6PDase) and glucose-6-phosphatase (G6Pase), the liver was homogenized in 0·25 M-sucrose in a glass-teflon tube. G6PDase activity was assayed in the 105,000 g supernatant by the method described by Glock & McLean (1953), as modified by Löhr & Waller (1974), and G6Pase activity was determined in the microsomal fraction (Baginski, Foá & Zak, 1974). For mixed-function oxygenase assays, liver was homogenized in 1·15% KCl and aminopyrine demethylase and aniline hydroxylase activities were determined (Kato & Gillette, 1965). Protein contents of the different subcellular fractions of liver were estimated by the method of Lowry, Rosebrough, Farr & Randall (1951).

Brain cholinesterase. The activity of brain cholinesterase was determined by the method of Ellmann, Courtney, Andres & Featherstone (1961) as modified by Simon & Winter (1976).

Statistical analyses. Data were analysed for statistical significance by the Student's *t* test.

RESULTS

The rate of body-weight gain for both the male and female groups of rats receiving both methomyl and ethanol was considerably lower than that for the other groups, the response of the female rats being the more marked. The mean weight of the male rats became significantly different from that of the controls from week 4 ($P < 0.01$ at week 4 and < 0.001 at week 6) whereas the weight of the females treated with methomyl and ethanol was reduced significantly ($P < 0.001$) from week 2 compared with the controls (Fig. 1). Since these groups were pair-fed, the reduced growth was probably attributable to a decrease in food utilization.

The fluid consumption of both sexes treated with methomyl and ethanol was appreciably lower than that of the controls, the difference being more marked in the females (Fig. 2). Similar results were obtained if the calculations were based on the total fluid intake (fluid consumed plus fluid added to the diet; Table 1). Although the fluid consumption varied among the various groups no changes were seen in the terminal haematocrits (Table 2).

The mean daily methomyl and ethanol consumptions were calculated from the food and fluid intakes (Table 1). The lower ethanol intake by the methomyl/ethanol-treated females compared with those given only ethanol was accounted for by the lower total fluid intake of the former group.

The relative weights of adrenals in both males and

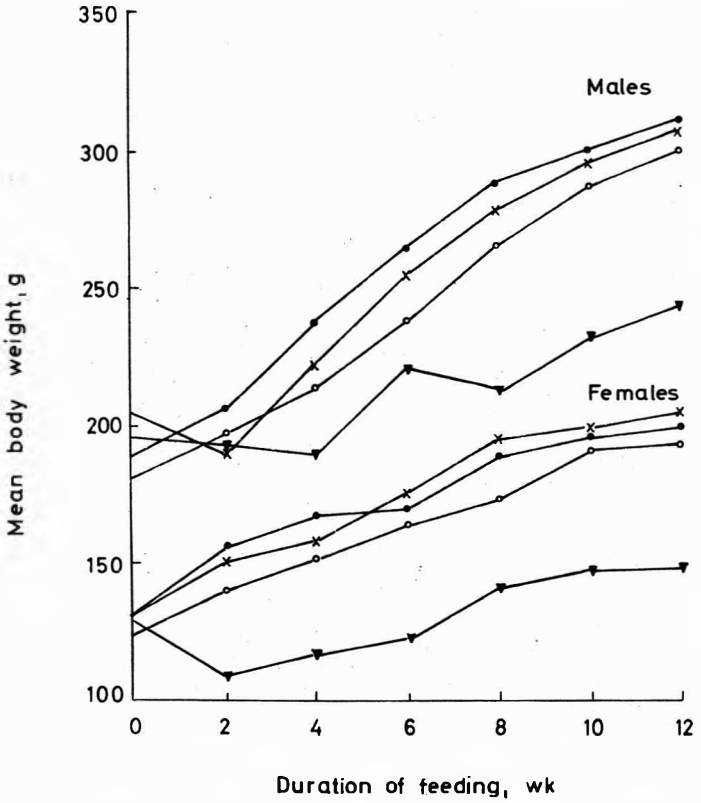

Fig. 1. Growth curves of groups of rats given, for 12 wk, tap-water and either a semi-synthetic control diet (●) or diet containing 200 mg methomyl/kg (○), 10% ethanol as drinking-fluid with either the control diet (×) or the methomyl diet (▼).

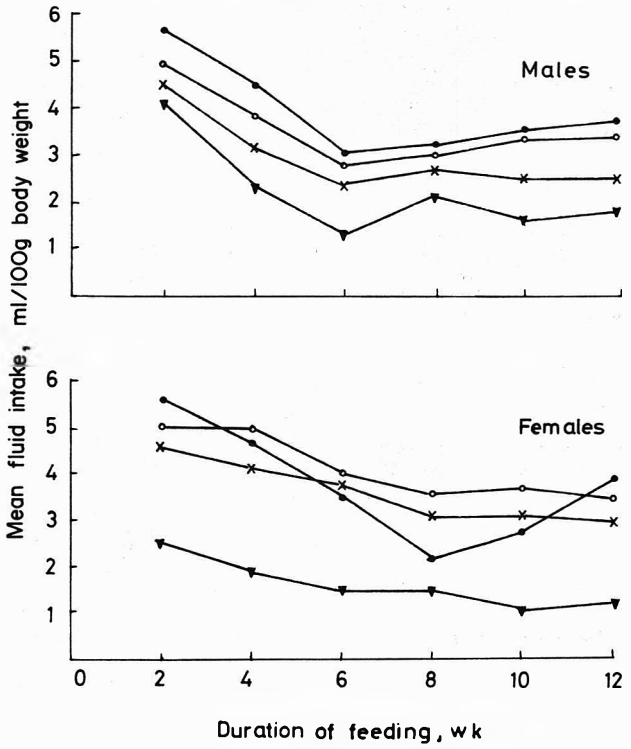

Fig. 2. Intake of drinking-fluid by groups of rats given tap-water and either a semi-synthetic control diet (●) or diet containing 200 mg methomyl/kg (○), or 10% ethanol as drinking-fluid with either the control diet (×) or the methomyl diet (▼).

females of the methomyl/ethanol-treated group were significantly higher than those for the other groups (Table 3). In the males the relative weights of the spleen were augmented by ingestion of ethanol or methomyl separately, but no further significant increase occurred in response to combined administration. The relative weight of the liver was significantly increased in the female rats given ethanol or methomyl plus ethanol, and the relative weight of the kidney was increased in females by the separate administration of the two agents and was increased further by the combined administration (Table 3). No pathological changes were seen in either organ by light microscopy.

The fasting blood-glucose levels were similar in all the male groups, whereas in the females ethanol treatment resulted in a lower value and the methomyl plus ethanol treatment was followed by a significantly higher fasting blood-glucose level than those in controls (Table 2). No appreciable differences were seen in hepatic glycogen among the various groups (Table 4). The triglyceride content of the liver was significantly higher than the control level in the methomyl-treated males and was further increased follow-

Table 1. *Mean daily fluid and food consumption of rats treated with methomyl and/or ethanol*

Group	Total fluid intake* (ml/100 g body weight/day)	Food consumption* (g/100 g body weight/day)	Methomyl intake (mg/100 g body weight/day)	Ethanol intake (g/100 g body weight/day)
		Males		
Control	6·19	5·15	—	—
Methomyl	6·60	5·41	1·08	—
Ethanol	5·73	5·44	—	0·46
Methomyl/ethanol	5·25	5·70	1·14	0·42
		Females		
Control	7·70	6·15	—	—
Methomyl	7·60	6·45	1·29	—
Ethanol	7·20	6·00	—	0·57
Methomyl/ethanol	5·52	6·42	1·28	0·44

*Total fluid (drinking fluid and dietary diluent) and food consumption values are those recorded at week 12 and are the means for groups of ten animals.

Table 2. *Effect of ingestion of methomyl and/or ethanol for 12 wk on the haematocrit and blood-glucose levels of rats*

Group	Haematocrit (%)	Glucose (mg/100 ml)
	Males	
Control	49·7 ± 1·47	107 ± 8·1
Methomyl	48·5 ± 1·72	116 ± 10·7
Ethanol	48·8 ± 1·48	111 ± 14·3
Methomyl/ethanol	49·0 ± 1·73	109 ± 8.3
	Females	
Control	49·0 ± 1·59	96 ± 9·1
Methomyl	51·1 ± 1·91	83 ± 7·9
Ethanol	51·6 ± 1·57	68 ± 14·3**
Methomyl/ethanol	51·5 ± 2·00	127 ± 24·9**

Values are expressed as the means ± SD for groups of ten rats, and those marked with asterisks differ significantly (Student's *t* test) from the control value: $^{**}P < 0{\cdot}01$.

ing the combined administration. The free fatty acid content was decreased by methomyl but increased significantly by combined administration of methomyl and ethanol. No changes in lipid metabolism were apparent in the treated female rats (Table 4).

G6PDase activity was reduced significantly in the livers of methomyl-treated male and female rats (Table 5). The effect of methomyl appeared to account for a similar depression of G6PDase activity in the methomyl/ethanol-treated groups. Microsomal G6Pase activity was increased only in the females treated with ethanol or with methomyl plus ethanol. Of the drug-metabolizing enzymes tested, only aniline hydroxylase was increased both in males and females given ethanol or methomyl plus ethanol (Table 5). The increases in both of these enzymes following the combined treatment appeared to be attributable to the ethanol (Table 5).

Brain cholinesterase activity was decreased in the male rats by administration of methomyl or ethanol and a further reduction occurred on combined admini-

Table 3. *Relative organ weights of rats treated with methomyl and/or ethanol for 12 wk*

Group	Relative organ weight (g/100 g body weight)				
	Liver	Kidneys	Adrenals‡	Heart	Spleen‡
			Males		
Control	3·58 ± 0·41	0·78 ± 0·12	15·21 ± 0·94	0·32 ± 0·03	97·00 ± 11·46
Methomyl	3·73 ± 0·81	0·80 ± 0·12	15·26 ± 1·82	0·32 ± 0·05	118·90 ± 15·76**
Ethanol	3·81 ± 0·32	0·74 ± 0·03	15·50 ± 1·18	0·33 ± 0·03	116·90 ± 7·90**
Methomyl/ethanol	3·71 ± 0·22	0·87 ± 0·04	19·48 ± 2·15**	0·35 ± 0·03	124·00 ± 14·90**
			Females		
Control	2·90 ± 0·26	0·67 ± 0·03	24·60 ± 2·90	0·34 ± 0·03	159·40 ± 23·99
Methomyl	3·02 ± 0·18	0·74 ± 0·03**	25·63 ± 3·98	0·36 ± 0·03	164·00 ± 35·88
Ethanol	3·18 ± 0·12*	0·74 ± 0·04**	24·83 ± 3·37	0·36 ± 0·03	169·20 ± 23·88
Methomyl/ethanol	3·28 ± 0·32*	0·90 ± 0·07†	28·80 ± 2·64	0·37 ± 0·01	174·80 ± 38·86

‡Weights of these organs are expressed in mg/100 g body weight.

Values are expressed as the means ± SD for groups of ten rats, and those marked with asterisks differ significantly (Student's *t* test) from the control value: $^{*}P < 0{\cdot}05$; $^{**}P < 0{\cdot}01$. The value marked with an arrow differs significantly from the values from the methomyl- and ethanol-treated groups: $\dagger P < 0{\cdot}01$.

Table 4. *Hepatic triglyceride, free fatty acid and glycogen levels in rats treated with methomyl and/or ethanol for 12 wk*

Group	Triglyceride (mg/g liver)	Free fatty acid (μmol/g liver)	Glycogen (mg/g liver)
		Males	
Control	23·50 ± 4·87	33·90 ± 5·50	14·00 ± 0·06
Methomyl	33·00 ± 5·42*	27·39 ± 6·00*	14·00 ± 0·45
Ethanol	26·25 ± 4·78	38·08 ± 11·20	12·20 ± 0·60
Methomyl/ethanol	39·90 ± 10·40**	43·30 ± 2·89**	14·90 ± 0·97
		Females	
Control	32·13 ± 5·50	27·82 ± 3·94	15·02 ± 2·00
Methomyl	31·20 ± 7·70	27·28 ± 4·50	14·70 ± 1·80
Ethanol	30·28 ± 7·10	27·10 ± 5·30	14·10 ± 2·10
Methomyl/ethanol	34·93 ± 8·32	25·70 ± 5·20	14·00 ± 1·90

Values are expressed as the means ± SD for groups of ten rats, and those marked with asterisks differ significantly (Student's *t* test) from the control value: $^{*}P < 0{\cdot}05$; $^{**}P < 0{\cdot}01$.

Table 5. *Changes in the activities of certain hepatic enzymes in rats after treatment with methomyl and/or ethanol for 12 wk*

Group	Enzyme activities†			
	G6PDase	G6Pase	AHO	APDM
Males				
Control	0·088 ± 0·006	0·23 ± 0·02	0·009 ± 0·001	0·072 ± 0·014
Methomyl	0·062 ± 0·009**	0·26 ± 0·03	0·010 ± 0·002	0·064 ± 0·014
Ethanol	0·076 ± 0·015	0·25 ± 0·03	0·013 ± 0·002**	0·073 ± 0·005
Methomyl/ethanol	0·063 ± 0·008**	0·23 ± 0·02	0·013 ± 0·003**	0·070 ± 0·008
Females				
Control	0·144 ± 0·016	0·11 ± 0·02	0·006 ± 0·001	0·036 ± 0·012
Methomyl	0·105 ± 0·022***	0·12 ± 0·03	0·006 ± 0·001	0·034 ± 0·009
Ethanol	0·142 ± 0·018	0·18 ± 0·03**	0·008 ± 0·001*	0·031 ± 0·009
Methomyl/ethanol	0·094 ± 0·013***	0·19 ± 0·03**	0·009 ± 0·002**	0·041 ± 0·013

†G6PDase = Glucose-6-phosphate dehydrogenase (μmol NADPH/mg protein/min); G6Pase = glucose-6-phosphatase (μmol Pi/mg protein/min); AHO = aniline hydroxylase (μmol *p*-aminophenol/mg protein/hr); APDM = aminopyrine demethylase (μmol formaldehyde/mg protein/hr).
Values are expressed as the means ± SD for groups of ten rats and those marked with asterisks differ significantly (Student's *t* test) from the control value: $*P < 0·05$; $**P < 0·01$; $***P < 0·001$.

stration (Table 6). No such additive effect was seen with the females, in which a similar reduction in cholinesterase activity occurred in all the treated groups.

DISCUSSION

Our previous studies showed that methomyl interfered slightly with lipid metabolism. In the present investigations the dose of methomyl was chosen accordingly. Earlier results from this laboratory also showed that ethanol—the so-called second toxic agent—failed to influence metabolic functions in the liver of this strain of rat when offered as the drinking-fluid even in a concentration of 15%. The responses to treatment with either agent applied separately were essentially the same in the present studies as were observed previously. At the same time, results of the combined administration of methomyl and ethanol suggest that the actions of these two agents are additive in some respects. Although the animals were pair fed, it is notable that the rates of body-weight gain for both males and females given the combined treatment were significantly lower than for the other groups.

Table 6. *Changes in brain acetylcholinesterase activity in rats following ingestion of methomyl and/or ethanol for 12 wk*

Group	Brain acetylcholinesterase activity‡	
	Males	Females
Control	2·09 ± 0·06	1·93 ± 0·12
Methomyl	1·80 ± 0·04***	1·49 ± 0·04***
Ethanol	1·88 ± 0·14**	1·52 ± 0·27**
Methomyl/ethanol	1·36 ± 0·10†	1·46 ± 0·11***

‡Expressed as μmol acetylthiocholine iodide/mg brain/min. Values are expressed as the means ± SD for groups of ten rats, and those marked with asterisks differ significantly (Student's *t* test) from the control values: $**P < 0·01$; $***P < 0·001$. The value marked with an arrow differs significantly from those for the methomyl- and ethanol-treated groups: $†P < 0·001$.

The relative weights of adrenals were increased in both males and females given the two compounds. Mallov & Bloch (1956), using a higher ethanol dose than we did, were able to induce adrenal hypertrophy in a long-term study and suggested the involvement of the adrenal cortical hormones in the development of fatty liver. Under the experimental conditions now described, ethanol *per se* failed to influence the hepatic triglyceride content in the male rats while methomyl increased it, and a combination of the two agents resulted in a marked increase. It is possible that the adrenals are involved in the mechanism underlying this effect. The increase in the relative liver weight in both groups of female rats given ethanol was not considered to be of toxicological significance and, like the increase in relative kidney weights seen in all the treated females, it was not accompanied by any pathological changes detectable by light microscopy.

In well-fed humans and animals, ethanol is known to induce a slight and transient hyperglycaemia which has been attributed in part to the release of epinephrine. Ethanol ingestion also causes hypoglycaemia during starvation, by blocking gluconeogenesis (Lundquist, 1971), a point that may account for the lower fasting level of blood glucose in ethanol-treated female rats. The cause of the higher blood-glucose level in the females treated with methomyl plus ethanol remains to be elucidated, but the role of catecholamines is a factor to be taken into consideration.

Smyth, Martin, Moss & Beck (1967) have shown that the long-term administration of ethanol decreases brain-cholinesterase activity, and the same effect was observed with ethanol in the present study. The cholinesterase-inhibiting action of methomyl is well known. A striking finding in the work now reported, however, was the difference in the response of male and female rats to the combined administration of the two agents (Table 6). While the cholinesterase activity in the brain of male rats was significantly lower after the combined administration than

after the ingestion of either agent alone, no such additive action was seen in the females.

The results of this study indicate that ingestion of these two agents for 3 months may result in interactions, even when the ethanol is administered in a relatively low concentration.

REFERENCES

Baginski, E. S., Foá, P. P. & Zak, B. (1974). Methods for determination of enzyme activity. In *Methods of Enzymatic Analysis*. Edited by H. U. Bergmeyer. 2nd Ed. p. 876. Verlag Chemie, Weinheim Academic Press, Inc., New York, London.

Ellmann, G. L., Courtney, K. D., Andres, V. J. & Featherstone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmac.* **7**, 88.

Folch, J., Lees, M. & Sloane-Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *J. biol. Chem.* **226**, 497.

Glock, G. E. & McLean, P. (1953). Further studies on the properties and assay of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase of rat liver. *Biochem. J.* **55**, 400.

Good, C. A., Kramer, H. & Somogyi, M. (1933). The determination of glycogen. *J. biol. Chem.* **100**, 485.

Kato, R. & Gillette, J. R. (1965). Effect of starvation on NADPH-dependent enzymes in liver microsomes of male and female rats. *J. Pharmac. exp. Ther.* **150**, 279.

Löhr, G. W. & Waller, H. D. (1974). Methods for determination of enzyme activity. In *Methods of Enzymatic Analysis*. Edited by H. U. Bergmeyer. 2nd Ed. p. 636. Verlag Chemie, Weinheim Academic Press, Inc., New York-London.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193**, 265.

Lundquist, F. (1971). Influence of ethanol on carbohydrate metabolism. *Q. J. Stud. Alcohol* **32**, 1.

Mallov, S. & Bloch, J. L. (1956). Role of hypophysis and adrenals in fatty infiltration of liver resulting from acute ethanol intoxication. *Am. J. Physiol.* **184**, 29.

Mosinger, F. (1965). Photometric adaptation of Dole's microdetermination of FFA. *J. Lipid Res.* **6**, 157.

Simon, G. & Winter, M. (1976). The effect of sympatholytic and sympathomimetic agents on acetylcholinesterase and cholinesterase activity, in vitro. *Biochem. Pharmac.* **25**, 881.

Smyth, R. D., Martin, G. J., Moss, J. N. & Beck, H. (1967). The modification of various enzyme parameters in brain acetylcholine metabolism by chronic ingestion of ethanol. *Expl Med. Surg.* **25**, 1.

Varsányi Nagy, M. (1974). Serum triglicerid mérése. *Orvosi Hetilap* **115**, 453.

Fd Cosmet. Toxicol. Vol. 17, pp. 339 to 341

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FURTHER STUDIES ON THE CARCINOGENICITY OF A FOOD ADDITIVE, AF-2, IN HAMSTERS

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Abstract—The carcinogenicity of 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2), once used as a food additive, was studied in golden hamsters of both sexes by continuous oral administration for 660 days at a level of 0·08 or 0·16% in the basal diet. The first forestomach tumours were detected on days 498 and 659 in the males and on days 444 and 451 in the females of the groups fed 0·08 and 0·16% AF-2, respectively, the incidence at day 660 in these two groups being 65 and 100% in the males and 58 and 76% in the females, respectively. At day 660, squamous-cell carcinomas of the forestomach were found in 15 and 47% of males and 0 and 6% of females given 0·08 and 0·16% AF-2, respectively. Oesophageal tumours were found only in hamsters given 0·16% AF-2, the incidence being 35% in males and 6% in females. No tumours were found in other organs, except one phaeochromocytoma in the adrenal of a female given 0·08% AF-2. In the control groups, no tumours were observed in any organs examined.

INTRODUCTION

The nitrofuran derivative 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2) was used in Japan from 1965 to 1974 as a food preservative.

Towards the end of this period, AF-2 was found to be mutagenic in various biological systems (Kada, 1973; Kondo & Ichikawa, 1973; Ong & Shahin, 1974; Tazima & Onimaru, 1974; Tonomura & Sasaki, 1973; Yahagi, Matsushima, Nagao, Seino, Sugimura & Bryan, 1976; Yahagi, Nagao, Hara, Matsushima, Sugimura & Bryan, 1974). Its carcinogenicity was first observed in mice (Ikeda, Horiuchi, Furuya, Uchida, Suzuki & Azegami, 1974) and later confirmed in mice and rats (Cohen, Ichikawa & Bryan, 1977; Nomura, 1975; Takayama & Kuwabara, 1977a, b). We have already reported the carcinogenicity of AF-2 in hamsters (Sano, Kawachi, Matsukura, Sasajima & Sugimura, 1977), but in our study only a few male hamsters and one dose of AF-2 were used.

The present paper reports the carcinogenicity of AF-2 in golden hamsters of both sexes given a diet containing 0·08 or 0·16% AF-2 for 660 days.

EXPERIMENTAL

Test chemical. AF-2 was obtained through the Ministry of Health and Welfare, Japan, and was confirmed as 99·66% pure by NMR spectroscopy and thin-layer chromatography.

Animals and diet. Golden hamsters, aged 4 wk and weighing 60–65 g, were purchased from the Cooperative Society of Experimental Animals, Shizuoka. Basal diet, CE-2 (CLEA Japan Inc., Tokyo), untreated or supplemented with AF-2, was given to the animals in pelleted form.

Experimental design. Groups of 25 males and 25 females were given CE-2 containing either 0·08 or 0·16% AF-2, and control groups of 20 males and 20 females were given CE-2 diet without AF-2, for 660 days. The total amount of AF-2 consumed by each animal was estimated from the food intake, which was measured every 2 days.

Animals were killed when they became moribund or at the end of the experiment and were autopsied. The thoracic and abdominal organs were fixed in 15% neutral formalin solution. The upper alimentary tract, from the tongue to the duodenum, was removed *en bloc* and opened along the greater curvature of the stomach. Tissues were embedded in paraffin wax and sections were stained with haematoxylin and eosin for microscopic examination. The histological examinations were performed on all organs of all the 35 control and 71 treated animals that survived more than 444 days.

RESULTS

The gains in body weight were similar in AF-2-treated and control hamsters throughout the experiment.

The tumours found in each group are summarized in Table 1. No neoplastic changes were found in any organs of the 29 experimental hamsters and five controls that died of pneumonia before day 444. The first tumour was found in the forestomach of a female hamster fed on diet containing 0·08% AF-2 and dying on day 444 of the experiment.

Surviving hamsters were killed and examined on day 660, all the survivors being by that time in a poor state of health. Of the hamsters given 0·08% AF-2, 65% of the males and 58% of the females had forestomach tumours, three of those in the 13 affected males and none in the ten females being squamous-cell carcinomas. All of the surviving males and 76% of the surviving females given 0·16% AF-2 had forestomach tumours, eight of the 17 in the males and one of the 13 in the females being squamous-cell carcinomas.

Table 1. *Incidence of tumours in hamsters fed 0·08 or 0·16% AF-2 in the diet for up to 660 days*

Dietary AF-2 level (%)	Sex	Total AF-2 dose ingested (g)	No. of hamsters		No. of hamsters with tumours of		
			Initial	Effective*	Forestomach†	Oesophagus†	Other organs
0	M	0	20	19	0	0	0
	F	0	20	16	0	0	0
0·08	M	2·5	25	20	13 (3)	0	0
	F	2·4	25	17	10 (0)	0	1‡
0·16	M	5·3	25	17	17 (8)	6 (1)	0
	F	4·7	25	17	13 (1)	1 (0)	0

*Number of hamsters surviving on day 444, when the first tumour was found.
†Numbers in parentheses are numbers of hamsters with squamous-cell carcinoma.
‡Phaeochromocytoma of the adrenal.

No oesophageal tumours were found in hamsters fed on the 0·08% AF-2 diet, but they occurred in 35% of the males and one (6%) of the females given 0·16% AF-2. One of the six found in males was a squamous-cell carcinoma.

Grossly, single polyp or multiple polypoid tumours, 1–2 mm in diameter, were seen in the forestomach and oesophagus. There were less than ten tumours per forestomach in male and female hamsters given the 0·08% AF-2 diet and in females given the higher level, but there were more than ten per forestomach in some male hamsters fed the 0·16% AF-2 diet.

Histologically, the benign tumours in the forestomach and oesophagus were squamous-cell papillomas, showing slight cellular atypism without invasion below the basal layer. Squamous-cell carcinomas were composed of irregularly arranged atypical cells and keratin pearls. Most of those found in the forestomach invaded the submucosa and some invaded the muscularis propria or serosa. The squamous-cell carcinoma of the oesophagus found in a male hamster invaded the muscularis propria.

No metastases from the squamous-cell carcinomas were found and, with one exception, no tumours were observed in any other organs. The exception was a phaeochromocytoma in the left adrenal of a female hamster fed 0·08% AF-2. No neoplastic changes were observed in any organs of either male or female hamsters in the control groups. A high incidence of cyst formation in the liver, with dilatation of the bile duct, was found in all the experimental groups given AF-2.

DISCUSSION

This study shows that the food additive AF-2 induces carcinoma of the forestomach and oesophagus of hamsters, as described previously (Sano *et al.* 1977). The incidence of squamous-cell carcinoma of the forestomach in males at the two doses of AF-2 differed significantly ($P < 0·05$ by Student's t test). In female hamsters, squamous-cell carcinoma was observed only in animals given 0·16% AF-2 in the diet. At both dose levels the incidence of squamous-cell carcinoma differed significantly ($P < 0·001$ by Student's t test) between males and females.

AF-2 has been reported to induce tumours of the forestomach in CDF_1 mice and Wistar rats, the incidence of tumours being correlated with the dose of AF-2. In female Wistar rats, AF-2 induced tumours mainly in the mammary gland, while in male Wistar rats, it induced skeletal muscle atrophy (Takayama & Kuwabara, 1977a, b). No mammary tumours or skeletal muscle atrophy were observed in hamsters in this study.

Man is exposed to a very large number of artificial and natural environmental carcinogens (Tomatis, Agthe, Bartsch, Huff, Montesano, Saracci, Walker & Wilbourn, 1978), and thus it is essential to estimate the carcinogenic potencies of compounds suspected of being human hazards. Formerly AF-2 was produced commercially and used widely as a food additive in Japan, so it became necessary to assess its risk to man. The potency of carcinogens can be expressed as the TD_{50}, defined as the daily dose/kg body weight that, when fed over a standard life-time, induces tumours in half of the animals tested (Hooper, Friedman, Sawyer & Ames, 1977). It is difficult to estimate human risk from the results of animal experiments, but quantitative analysis is necessary for determining the potency of carcinogens and assessing this human risk. The TD_{50} of AF-2 for tumours of the forestomach of hamsters is calculated as 23 and 25 mg/kg/day in the males and females fed 0·08% AF-2 and 18 and 30 mg/kg/day in those fed 0·16% AF-2, respectively, the average body weight being 123, 133, 129 and 131 g for these four groups. The total intakes of AF-2 were 2·5, 2·4, 5·3 and 4·7 g, respectively, as shown in Table 1. In Japan, about 2·7 metric tons AF-2 was produced each year from 1965 to 1974. From this, the daily intake of AF-2 per head can be estimated as less than 0·012 mg/kg body weight, taking the average body weight as 60 kg and the population of Japan as 100 million. In comparison, this dose is one two thousandth of the average TD_{50} in hamsters, 24 mg/kg/day.

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REFERENCES

Cohen, S. M., Ichikawa, M. & Bryan, G. T. (1977). Carcinogenicity of 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2) fed to female Sprague-Dawley rats. *Gann* **68**, 473.

Hooper, N. K., Friedman, A. D., Sawyer, C. B. & Ames, B. N. (1977). Carcinogenic potency: Analysis, utility for human risk assessment, and relation to mutagenic potency in *Salmonella*. Progress Report for IARC/WHO Meeting, October 3–7.

Ikeda, Y., Horiuchi, S., Furuya, T., Uchida, O., Suzuki, K. & Azegami, J. (1974). Induction of gastric tumors in mice by feeding of furylfuramide (in Japanese). Food Sanitation Study Council, Ministry of Health and Welfare, Japan.

Kada, T. (1973). *Escherichia coli* mutagenicity of furylfuramide. *Jap. J. Genet.* **48,** 301.

Kondo, S. & Ichikawa, R. (1973). Testing and classification of mutagenicity of furylfuramide in *Escherichia coli*. *Jap. J. Genet.* **48,** 295.

Nomura, T. (1975). Carcinogenicity of the food additive furylfuramide in foetal and young mice. *Nature, Lond.* **258,** 610.

Ong, T. & Shahin, M. M. (1974). Mutagenic and recombinogenic activities of the food additive furylfuramide in eukaryotes. *Science, N.Y.* **184,** 1086.

Sano, T., Kawachi, T., Matsukura, N., Sasajima, K. & Sugimura, T. (1977). Carcinogenicity of a food additive, AF-2, in hamsters and mice. *Z. Krebsforsch.* **89,** 61.

Takayama, S. & Kuwabara, N. (1977a). Carcinogenic activity of 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide, a food additive, in mice and rats. *Cancer Lett.* **3,** 115.

Takayama, S. & Kuwabara, N. (1977b). The production of skeletal muscle atrophy and mammary tumors in rats by feeding 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide. *Toxicology Lett.* **1,** 11.

Tazima, Y. & Onimaru, K. (1974). Results of mutagenicity testing for some nitrofuran derivatives in a sensitive test system with silkworm oocytes. *Mutation Res.* **26,** 440.

Tomatis, L., Agthe, C., Bartsch, H., Huff, J., Montesano, R., Saracci, R., Walker, E. & Wilbourn, J. (1978). Evaluation of the carcinogenicity of chemicals: A review of the monograph program of the International Agency for Research on Cancer (1971 to 1977). *Cancer Res.* **38,** 877.

Tonomura, A. & Sasaki, M. S. (1973). Chromosome aberration and DNA repair synthesis in cultured human cells exposed to nitrofurans. *Jap. J. Genet.* **48,** 291.

Yahagi, T., Matsushima, T., Nagao, M., Seino, Y., Sugimura, T. & Bryan, G. T. (1976). Mutagenicities of nitrofuran derivatives on a bacterial tester strain with an R factor plasmid. *Mutation Res.* **40,** 9.

Yahagi, T., Nagao, M., Hara, K., Matsushima, T., Sugimura, T. & Bryan, G. T. (1974). Relationships between the carcinogenic and mutagenic or DNA-modifying effects of nitrofuran derivatives, including 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide, a food additive. *Cancer Res.* **34,** 2266.

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TERATOLOGY STUDIES IN MICE EXPOSED TO MUNICIPAL DRINKING-WATER CONCENTRATES DURING ORGANOGENESIS

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Abstract—Organic materials concentrated from the drinking-waters of five US cities selected as representative of the major sources of raw water and a sample of low-molecular-weight organohalides were administered to groups of pregnant CD-1 mice on gestation days 7–14 by oral intubation. Each of the six test materials was dissolved in dimethylsulphoxide (DMSO), and dosage levels represented 3000, 1000 and 300 times the anticipated human exposure to these materials. The dams were killed on day 18 of gestation, and the foetuses were examined for skeletal and visceral anomalies. No effects in the foetus were attributable to the administration of DMSO or to the organic materials from the municipal drinking-waters. It was concluded that the organic impurities present in a wide sample of municipal drinking-waters possess very little capacity for inducing foetotoxicity in the mouse.

INTRODUCTION

It has been estimated that over 1296 organic compounds have been identified in sources of water (Shackelford & Keith, 1976). Almost 700 organic contaminants have been identified in drinking-waters in the United States (Environmental Protection Agency, 1978) and little is known about their health effects (Garrison, 1977). The report of a higher incidence of resorption and malformations (principally cleft palate) in mice drinking tap-water from Durham, NC, than in those drinking purified water (McKinney, Maurer, Hass & Thomas, 1976) prompted much concern over the possible effects that contaminants present in drinking-water might have on reproductive parameters. The authors, however, could not implicate microparticulate or dissolved organic or inorganic materials in the toxic response.

Later studies using a similar experimental protocol (Staples, Worthy & Marks, 1979; Chernoff, Rogers, Carver, Kavlock & Gray, 1979) produced no evidence that the type of water affected either the incidence of resorptions or of malformations, but Chernoff *et al.* (1979) found an increased incidence of supernumerary ribs in the tap-water group and both studies reported considerable month-to-month fluctuations in the data. These changes favoured the tap-water as often as the purified-water group. For example, the latter authors reported that the incidence of foetal resorptions for the month of November was 9·3% in the purified-water group and 18·3% in the tap-water group, and in December the relationship was reversed (24·2% versus 13·0%). These studies were hampered by the use of only one dose and by the problems inherent in detecting significant alterations in development with limited numbers of test animals at levels of environmental exposure.

The current study is part of a larger investigation (Tardiff, Carlson & Simmon, 1976) designed to evaluate the potential toxicity of drinking-water impurities from various cities. Concentration of these impurities made possible the use of multiple dose levels, a decided advantage over previous studies.

EXPERIMENTAL

Animals. CD-1 mice, 60 days old, were obtained from the Charles River Breeding Laboratory (Wilmington, MA). Males were housed singly and females four to a cage. All animals received purified drinking-water (prepared in a Corning "Meg Pure" glass still equipped with a Corning 3508-ORC organic removal cartridge and a Corning 3508-B demineralizer) and Purina Lab Chow (Ralston-Purina Co., Inc., St Louis, MO) *ad lib.* After a 1-wk acclimatization period, females were bred by placing them overnight with a male. The morning that a vaginal plug was found was designated day 1 of gestation. The inseminated females were then randomly assigned to 1 of the 20 treatment groups.

Materials. Organic materials were obtained from the drinking-waters of five cities using the reverse osmosis method described by Kopfler, Coleman, Melton & Tardiff (1977). Such water supply systems represent the major types of raw-water sources in the United States (Tardiff *et al.* 1976). The cities (together with the type of raw water used in generating drinking-water) were: Miami, FL (ground water receiving no known contamination), Seattle, WA (surface water receiving no known contamination), New Orleans,

Table 1. *Effects of exposure of CD-1 mice to drinking-water during organogenesis*

Treatment group	Daily dose†	Conception‡	Material weight gain§ (g)	Maternal liver weight (% of body weight)	Mean implantations per litter	Mean foetal data				
						Mortality (%)	Weight (g)	No. of sternebrae	No. of caudal ossification centres	Incidence of extra ribs (%)
Untreated control	0	72/84	5·2 ± 0·2	7·0 ± 0·1	11·9 ± 0·4	13·3 ± 2·9	1·00 ± 0·01	5·6 ± 0·1	3·4 ± 0·1	31 ± 4
DMSO control	0·1 ml	69/82	4·8 ± 0·3	6·9 ± 0·1	11·9 ± 0·4	13·6 ± 1·5	1·00 ± 0·01	5·6 ± 0·1	3·4 ± 0·1	23 ± 3
New Orleans	300	15/18	5·1 ± 0·7	6·5 ± 0·1	13·0 ± 0·4	9·4 ± 2·3	0·90 ± 0·02	4·9 ± 0·2	2·8 ± 0·2	24 ± 6
	1000	13/19	2·5 ± 0·7	7·1 ± 0·2	13·4 ± 1·0	12·6 ± 3·1	0·97 ± 0·02	5·3 ± 0·3	3·2 ± 0·2	20 ± 7
	3000	17/20	4·5 ± 0·4	6·6 ± 0·1	11·9 ± 0·8	11·6 ± 2·2	0·96 ± 0·01	5·3 ± 0·2	3·1 ± 0·2	29 ± 7
Miami	300	11/20	4·8 ± 0·6	6·5 ± 0·2	12·4 ± 0·7	13·4 ± 4·0	0·97 ± 0·03	5·3 ± 0·3	3·4 ± 0·3	24 ± 11
	1000	17/20	4·9 ± 0·3	6·7 ± 0·1	11·9 ± 0·8	12·4 ± 2·4	0·95 ± 0·03	5·6 ± 0·1	3·5 ± 0·2	25 ± 6
	3000	15/20	4·6 ± 0·7	6·8 ± 0·1	12·9 ± 0·7	9·7 ± 2·3	0·96 ± 0·02	5·6 ± 0·1	3·6 ± 0·2	19 ± 7
Philadelphia	300	11/19	4·1 ± 0·6	6·8 ± 0·1	12·4 ± 0·7	6·7 ± 2·2	0·98 ± 0·01	5·5 ± 0·1	3·3 ± 0·2	28 ± 8
	1000	12/20	4·5 ± 0·6	6·7 ± 0·2	12·2 ± 0·9	11·9 ± 3·5	0·97 ± 0·03	5·5 ± 0·1	3·4 ± 0·2	20 ± 9
	3000	18/20	4·5 ± 0·5	6·7 ± 0·1	11·7 ± 0·9	13·6 ± 3·2	0·97 ± 0·02	5·4 ± 0·1	3·3 ± 0·2	29 ± 7
Seattle	300	15/20	6·0 ± 0·5	7·0 ± 0·1	10·9 ± 0·7	10·1 ± 2·8	0·88 ± 0·02	4·7 ± 0·4	2·5 ± 0·3	27 ± 8
	1000	14/20	5·6 ± 0·4	7·1 ± 0·2	12·7 ± 0·9	14·4 ± 2·4	0·94 ± 0·01	5·4 ± 0·2	3·1 ± 0·2	26 ± 8
	3000	16/20	5·4 ± 0·3	7·4 ± 0·1**	12·5 ± 0·5	10·4 ± 2·2	0·98 ± 0·02	5·7 ± 0·2	3·6 ± 0·2	29 ± 7
Ottumwa	300	16/20	5·7 ± 0·5	7·3 ± 0·2	11·4 ± 0·4	7·9 ± 2·4	0·96 ± 0·02	5·5 ± 0·1	3·3 ± 0·2	34 ± 9
	1000	12/20	5·7 ± 0·3	7·5 ± 0·2	12·6 ± 0·4	8·1 ± 1·5	0·99 ± 0·02	5·6 ± 0·2	3·4 ± 0·3	18 ± 9
	3000	18/20	6·3 ± 0·4**	7·3 ± 0·1	13·2 ± 0·4	8·7 ± 2·2	0·99 ± 0·02	5·9 ± 0·1	3·7 ± 0·1	27 ± 8
Volatile fraction	300	18/29	5·7 ± 0·3*	7·3 ± 0·2	12·3 ± 0·5	7·3 ± 1.9	0·95 ± 0·02	5·6 ± 0·1	3·2 ± 0·2	19 ± 5
	1000	20/30	5·9 ± 0·4*	7·5 ± 0·2	13·1 ± 0·5	11·5 ± 2·4	0·94 ± 0·02	5·4 ± 0·1	3·2 ± 0·1	24 ± 4
	3000	25/30	6·3 ± 0·3**	7·3 ± 0·1	11·1 ± 0·6	18·6 ± 4·1	0·97 ± 0·03	5·0 ± 0·3	3·0 ± 0·3	23 ± 6

†For the test materials, the dose/pregnant mouse/day is expressed as a multiple of the estimated level of human exposure.
‡No. pregnant/no. mated.
§(Weight on day 18 − gravid uterus weight) − weight on day 6.
Values are means ± SEM and those marked with asterisks differ significantly from the DMSO-control values: *$P < 0·05$; **$P < 0·01$.

LA (surface water receiving industrial wastes), Ottumwa, IO (surface water receiving agricultural run-off), and Philadelphia, PA (surface water receiving municipal wastes).

Because organohalides with molecular weights less than approximately 200 are lost during the concentration procedure (Kopfler *et al.* 1977), a synthetic mixture (volatile fraction) of these compounds was prepared on the basis of the concentration of these materials in the drinking-waters of 110 cities, as determined by the National Organics Monitoring Survey conducted by the Technical Support Division, Office of Drinking Water, US Environmental Protection Agency, Washington, DC 20460. Since some compounds were found only in a few samples, the composition of the mixture was adjusted by multiplying the mean concentration from the cities where the compound was detected by the percentage of positive samples. By weight, the mixture contained the following compounds: chloroform (68·9%), bromodichloromethane (16·4%), dibromochloromethane (10·0%), bromoform (3·6%), 1,2-dichloroethane (0·01%), 1,1,1,-trichloroethane (0·04%), carbon tetrachloride (0·4%), trichloroethylene (0·2%), tetrachloroethylene (0·07%), benzene (0·2%), *o*-dichlorobenzene (0·09%), *m*-dichlorobenzene (0·001%) and *p*-dichlorobenzene (0·02%).

Dose levels. Dose levels in this study were based on multiples of expected human exposure levels. For the purpose of these calculations, the concentration of organic material in these drinking-waters was assumed to be 5 mg/litre for Miami, Philadelphia, Ottumwa and New Orleans, and 2 mg/litre for Seattle, with the synthetic volatile fraction at 100 μg/litre (F. Kopfler, personal communication 1977). It was assumed in the calculations that the average human weighed 60 kg and consumed 2 litres of water per day. Thus, human exposure to these organic materials in Miami would be given approximately by (2 litres/day) × (5 mg/litre)/(60 kg) = 0·17 mg/kg/day. Using factors of 300, 1000 and 3000 times the human exposure levels, mice receiving the Miami concentrate were exposed to 51, 170 or 510 mg/kg/day. Similar calculations were used for the other four samples.

Treatment. The concentrates and volatile fraction were dissolved in dimethylsulphoxide (DMSO) and administered by oral intubation on gestation days 7–14. The duration of exposure was limited by the small quantities of material available, and therefore did not encompass all of what is normally called organogenesis. Maternal weight on day 1 was used for calculation of the doses. Intubation volume was 0·1 ml/day. Control animals received the vehicle alone during the period of dosing. Because of reported DMSO-induced teratogenesis in hamsters (Ferm, 1966), a control group receiving sham intubations was included in the design of the study. All females were observed for any signs of overt central nervous system toxicity (salivation, tremors, convulsions, paralysis, ataxia etc.) during the period of administration.

Foetal examination. The inseminated mice were killed by decapitation on day 18 of gestation. The uterus was examined *in situ* for the presence of resorption sites and then removed and weighed. Maternal weight differences during gestation were calculated as the difference between the maternal weight on day 6 and on day 18 of gestation minus the gravid uterus weight. The maternal liver was removed and weighed and the liver/body weight percentage was calculated. The live foetuses were weighed and randomly distributed to either Bouin's solution (one half of the litter) or 65% alcohol (the other half). All foetuses were examined for external defects. An autopsy was performed on those fixed in Bouin's solution. Those in alcohol were cleared in 1% KOH, stained with Alizarin Red S and examined for skeletal development.

Statistics. Calculations were based upon the litter as the experimental unit. Data are presented in the tables in terms of the mean ± SEM. Statistical tests utilized in this study included Jonckheere's test (Jonckheere, 1954) for dose-response analysis, the Mann–Whitney *U* test for pairwise comparisons, and Fisher's Exact test for comparison of individual types of terata (Sokal & Rohlf, 1969).

RESULTS

Oral administration of 0·1 ml DMSO/day (approximately 3·3 g/kg/day) during days 7–14 of gestation failed to affect any parameter of maternal or foetal health when compared to sham-intubated controls (Tables 1 & 2). Visceral abnormalities were limited to two exencephalic foetuses with open eyes, one meningoencephalocele and one case of undescended testes in the sham-intubated group, compared with isolated occurrences of a meningoencephalocele, a cleft palate and open eyes in the DMSO-treated group.

Administration of any of the six test materials to pregnant mice at doses up to 3000 times the expected human-exposure levels produced few discernible effects on maternal health. No animal on any dosage regimen died during the course of the study and there was no indication of overt central nervous system toxicity in any animal on any dosage regimen. Statistically significant increases in maternal weight gain ($P < 0·01$) were noted in the groups given the Ottumwa concentrate and the volatile fraction and the liver weight expressed as a percentage of body weight showed a dose-related increase in the Seattle group ($P < 0·01$). No dosage regimen had any significant effects on foetal weight, mortality or sternal and caudal ossification centres, or on the occurrence of supernumerary ribs in the foetuses. The occurrences of visceral abnormalities in the treated groups were more than matched by defects observed in the control animals.

DISCUSSION

Previous studies on the possible foetotoxic potential of municipal drinking-waters (Chernoff *et al.* 1979; McKinney *et al.* 1976; Staples *et al.* 1979) have been conducted on water from only one source (Durham, NC) and the use of ambient concentrations as the sole dose level made the results difficult to assess. Chernoff *et al.* (1979) and Staples *et al.* (1979) examined the problem in some detail and failed to detect any effects other than, in the former study, an increased incidence of supernumerary ribs in the tap-water group. Such results are not totally unexpected when ambient levels of a compound or mixture are tested in small numbers of laboratory animals.

Table 2. *Occurrence of anomalies in foetal mice exposed to drinking-water concentrates during development*

Treatment group	Daily dose*	No. of litters examined	No. of foetuses autopsied	No. of foetuses stained with alizarin	Incidence† of						
					Exencephaly	Encephalocele	Micrognathia	Cleft palate	Undescended testes	Open eyes	Wavy ribs
Untreated control	0	72	354	387	2/2	1/1	0	0	1/1	2/2	0
DMSO control	0·1 ml	69	329	360	0	1/1	0	1/1	0	1/1	0
New Orleans	300	15	81	90	0	0	0	0	0	0	0
	1000	13	61	78	1/1	0	0	0	0	0	0
	3000	17	86	92	0	0	0	0	0	0	0
Miami	300	11	58	63	0	1/1	0	0	0	2/2	0
	1000	17	83	93	0	0	0	0	1/1	0	0
	3000	15	83	92	0	0	1/1	0	0	0	0
Philadelphia	300	11	60	66	0	0	0	0	0	0	1/1
	1000	12	61	69	0	0	0	0	0	0	0
	3000	18	88	93	0	0	0	0	0	0	0
Seattle	300	15	68	77	0	0	0	0	0	0	0
	1000	14	74	80	0	0	0	0	0	0	0
	3000	16	84	120	0	1/3	0	0	0	1/3	0
Ottumwa	300	16	80	87	0	0	0	0	0	0	0
	1000	12	67	71	0	0	0	0	0	0	0
	3000	18	103	113	0	0	0	0	0	0	0
Volatile fraction	300	18	99	101	0	0	0	0	0	0	0
	1000	20	110	120	0	0	0	0	0	0	0
	3000	25	109	120	0	1/1	0	0	0	0	0

*For the test materials, the dose/pregnant mouse/day is expressed as a multiple of the estimated level of human exposure.
†Positive findings are expressed as no. of litters affected/no. of foetuses affected.

This current study expanded the scope of the assessment of possible adverse effects on reproduction attributable to contaminants in drinking-waters by using contaminants from five water sources which were concentrated by a process of reverse osmosis. The results indicated that the organic impurities present in a wide sampling of municipal drinking-waters possess a very low capacity for inducing foetotoxicity. Doses of concentrates from Miami, FL, New Orleans, LA, Ottumwa, IO, Seattle, WA, and Philadelphia, PA, as high as 3000 times the expected levels of human exposure had no discernible effects on the foetal development of mice. In particular, there was no evidence of an increased incidence of foetal resorptions, cleft palate or supernumerary ribs as was reported in the previous water studies. Similarly, a composite sample of the lower-molecular-weight organic impurities that were lost during the concentration process failed to affect foetal development. The mice given the synthesized volatile fraction in doses 3000 times the anticipated human exposure received 10·3 mg/kg/day of the impurities, the majority of this (7·1 mg/kg/day) being chloroform. The lack of positive effects from this fraction is in agreement with the findings of Thompson, Warner & Robinson (1974) who intubated rats and rabbits during gestation and found effects only at doses above 50 mg/kg/day in rabbits and 126 mg/kg/day in rats.

REFERENCES

Chernoff, N., Rogers, E., Carver, B., Kavlock, R. & Gray, E. (1979). The fetotoxic potential of municipal drinking water in the mouse. *Teratology* **19**, 165.

Environmental Protection Agency (1978). Organic Compounds Identified in U.S. Drinking Waters. 1 April 1978. Health Effects Research Laboratory, Field Studies Division, Cincinnati, OH 45268.

Ferm, V. H. (1966). Teratogenic effect of dimethyl sulphoxide. *Lancet* **I**, 208.

Garrison, A. W. (1977). Analysis of organic compounds in water to support health effects studies. *Ann. N.Y. Acad. Sci.* **298**, 2.

Jonckheere, A. R. (1954). A distribution-free-K-sample test against ordered alternatives. *Biometrika* **41**, 333.

Kopfler, F. C., Coleman, W. E., Melton, R. G. & Tardiff, R. G. (1977). Extraction and identification of organic micropollutants: Reverse osmosis method. *Ann. N.Y. Acad. Sci.* **298**, 20.

McKinney, J. D., Maurer, R. R., Hass, J. R. & Thomas, R. O. (1976). Possible factors in the drinking water of laboratory animals causing reproductive failure. In *Proceedings of the Symposium on Identification and Analysis of Organic Pollutants in Waters.* First Chemical Congress of North America, Mexico City, Mexico.

Shackelford, W. & Keith, W. (1976) Frequency of organic compounds identified in water. Research Report EPA 600/4-76-062. Environmental Protection Agency, Washington, DC.

Sokal, R. & Rohlf, R. R. (1969). *Biometry.* p. 593. W. H. Freeman and Co., San Francisco, CA.

Staples, R. E., Worthy, W. C. & Marks, T. A. (1979). Influence of drinking water—tap verses purified—on embryo development in mice. *Teratology* **19**, 237.

Tardiff, R. G., Carlson, G. P. & Simmon, V. F. (1976). Halogenated organics in tap water: A toxicological evaluation. In *Proceedings of the Conference on the Environmental Impact of Water Chlorination.* Edited by R. L. Jolley. p. 213. NTIS CONF-751096.

Thompson, D. J., Warner, S. D. & Robinson, V. B. (1974). Teratology studies on orally administered chloroform in the rat and rabbit. *Toxic. appl. Pharmac.* **29**, 348.

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CONTAMINATION FROM SKIN-PAINTING TEST CHEMICALS

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Abstract—Anthracene, a non-carcinogenic polycyclic aromatic hydrocarbon, dissolved in acetone was painted on the clipped dorsal skin of mice in a laminar flow hood. Samples were taken over a 17-day period to estimate worker exposures to, and environmental contamination from, the chemicals used. The results indicated that the laminar flow hood retained nearly all the test chemical and the solvent, and that animal maintenance procedures and transport accounted for most of the contamination that occurred.

INTRODUCTION

The potential hazards from chemicals which may come into contact with human skin, either deliberately, as with a cosmetic, or inadvertently, via an occupational exposure, are often estimated by painting the chemical onto the skin of laboratory animals. The individuals who do this work may be exposed to the chemical being tested and/or the volatile solvent in which the chemical is usually dissolved.

A previous report (Darlow, Simmons & Roe, 1969) described the spread of *Bacillus globigii* spores applied to the clipped dorsal skin of mice. The investigators found that large numbers of spores became airborne during and immediately after application of the spore suspension, and as a result of changing bedding, reclipping the dorsal hair, and sweeping the floor. No estimates of worker exposures to acetone (the solvent in which the spores were suspended) were made. We believe that the potential exposures to the spores reported may be high for two reasons: *B. globigii* spores do not penetrate the skin and are therefore available for translocation or redispersion after their application to the skin; and the spores were applied to the skin in an unenclosed environment. (The authors observed, however, that preliminary work using rabbits showed that applying the spores within a safety cabinet eliminated the peak aerosol concentration associated with that operation.)

In this study, a noncarcinogenic polycyclic aromatic hydrocarbon (anthracene) dissolved in acetone was painted onto the clipped dorsal skin of mice in a laminar flow hood. Samples were taken over a 17-day period to estimate potential worker exposures to, and environmental contamination from, the chemicals used.

EXPERIMENTAL

Animals and conditions. Two rooms were used, one for animal housing and one for skin painting. Each room was about 3·6 m wide, 6·4 m deep, and 4·0 m high. The air supply to both rooms was via a louvered grille (15 × 91 cm) located above the door, 3 m (midline) above the floor. Airflow in both rooms was from the corridor to the room; the ventilation provided eight to ten air changes per hour. Air velocities in both rooms were variable but always less than 0·25 m/min. Dry bulb temperatures in the rooms ranged from 20–25°C and the relative humidity from 24–59% during the study.

All skin painting was done in a 2 m wide, Class II, Type B, laminar flow biological safety cabinet located in the skin-painting room. The hood face velocity was about 0·5 m/sec with the sash open to about 30 cm.

The tracer used was anthracene, $C_{14}H_{10}$, a noncarcinogenic (National Cancer Institute, 1977), fluorescent, polycyclic aromatic hydrocarbon.

One hundred and seventy five female BALB/cAnN mice (obtained from the Animal Production area of the Frederick Cancer Research Center), 6 wk old at the start of the experiment, were housed five to a cage. The polycarbonate cages (28 × 18 × 13 cm) were on one rack, which provided a spun glass filter-top, suspended cage system. Hardwood chip (Absorb-Dri, Garfield, NJ) bedding was used (c. 120 g/cage). An autoclaved granular diet (Wayne, Allied Mills, Inc., Chicago, IL) and water from glass bottles and stainless steel sipper tubes were available *ad lib.*

Treatment. Animals were brought into the housing room on day 1 of the study. The animals were acclimatized to environmental conditions from day 1–5. On day 6 the rack of animals was wheeled from the housing room to the skin-painting room.

For skin painting, one cage of mice at a time was placed in the hood. The dorsal hair of each mouse was removed with an electric clipper to expose a skin area approximately 1·5 × 2 cm. A 50 μl portion of a 1 mg anthracene/ml acetone solution was dropped onto the centre of the exposed dorsal skin of 150 of the mice using an automatic pipetting device (Eppendorf, Brinkman Instruments, Inc., Westbury, NY) with disposable pipette tips. The remaining 25 animals (controls) received 50 μl acetone administered

under identical conditions. The acetone was allowed to evaporate before the mice were removed from the hood. Animals were repainted on days 8, 10, 13, 15, 17, 20 and 22. Animals were reclipped as needed using surgical scissors which were thoroughly cleaned after each use. Animals were returned to the housing room after each painting was completed.

Mortality checks were done twice daily. Physical examinations, consisting of visual examination of the shorn dorsal area and light palpation, were performed on days 8, 10, 13, 15, 17 and 20. Cages and bedding were changed on days 6, 9, 13, 16 and 20. Water bottles were changed on days 6, 10, 13, 17 and 20. Feed was replenished as needed. At all other times, the animals were undisturbed. The floor of the housing room was wet mopped on days 6, 8, 10, 13, 15–17, 20 and 22.

Work Practices. Animal handlers wore a one-piece, zip-front, disposable Tyvek jumpsuit (a garment which covers the arms, legs, and trunk, and which has elastic to ensure a snug fit at the wrists and ankles, Dupont Co., Wilmington, DL), a gauze cap covering the hair and ears, and safety shoes covered by paper shoe covers. Latex gloves were worn for hand protection. When working in either room, the animal handler was required to wear a half-mask respirator fitted with organic vapour cartridges and prefilter pads. Prescription safety glasses or eye goggles were worn at all times. No eating, drinking, or smoking was permitted.

Workers were required to care for control animals before tending to treated animals.

All operations requiring moving the rack were carried out as carefully as possible to minimize contamination of the hallway between rooms. Water bottles were removed before moving the rack.

A stock solution of anthracene was prepared and stored in the dark at 4°C. The solution was stable under these conditions. When needed, aliquots were transported to the skin-painting room inside a secondary containment vessel.

All used pipette tips, chemical containers, and other debris were placed in a container in the hood for discarding. Hood surfaces were wiped with acetone, then detergent in water, and rinsed with water after each skin-painting operation.

Samples. Portions of the floor, walls, and other surfaces were wiped twice daily, before and after the work period, with a 9 cm diameter Whatman No. 1 filter paper (Whatman, Inc., Clifton, NJ) wetted with acetone. Wherever possible, a 15 × 15 cm area was wiped. Wipes from the mice, animal handler, equipment, and clothing were obtained at various times during the study. Some items such as clothes, bedding, and animal faeces were packaged for later evaluation.

Floor wipe samples were obtained twice a day in the animal housing room (25 data sets) and before and after skin painting in the hallway and the skin-painting room (eight data sets). The sampling locations are shown in Fig. 1.

Total suspended particulate matter was collected on 37 mm diameter Millipore type AA, membrane filters (0·8 μm pore size; Millipore Corp., Bedford, MA). In the animal-housing room the filter was suspended 1·7 m above the floor, about 15 cm in front of the animal rack. In the skin-painting room filters were located on each side of the worker 1·7 m above the floor. Sampling rates were about 18 litres/min. Samplers operated continuously in the animal-housing room, but only during skin-painting operations in the skin-painting room.

Respirable particulate matter concentrations were estimated by fitting the worker with a personal sampling pump (Model G, Mine Safety Appliances Co., Pittsburgh, PA) and 10 mm nylon cyclone upstream of a membrane filter. The sampling flow rate was 1·7 litres/min (AIHA Aerosol Technology Committee, 1970). Exposure to acetone vapour during skin-painting operations was estimated by fitting the worker with a pump (Model SP-1, Sipin Co., N.Y.) which drew air through a charcoal tube at about 50 ml/min. The tube was located in the worker's breathing zone.

Samples obtained during days 1–5 (the acclimatization period) were used to establish background levels of fluorescence.

Analysis. Samples were usually soaked in 20 ml of acetone. An aliquot was read for fluorescence on an Aminco-Bowman spectrophotofluorimeter manufactured by the American Instrument Co., Silver Spring, MD. Sample fluorescence was determined using an excitation wavelength of 375 nm, an emission wavelength of 426 nm, and a 1·0 mm phototube aperture. The relationship between fluorescence, expressed in arbitrary units (y), and concentration of anthracene, in μg/ml (x), was found to be $y = 8270x^{0.96}$. This relationship was established from the average of 18 pairs of standardization runs. The correlation coefficient of the data was more than 0·99. With this technique, 10 ng of anthracene could be reliably detected. Emission spectra of selected samples were obtained to verify that observed fluorescence was due to anthracene.

Adsorbed acetone vapour was eluted from charcoal tubes with ethyl acetate. The eluates were analysed on a gas chromatograph with a flame ionization detector (column: 2 m × 2 mm i.d. glass; packing: 0·2% SP 1000 on Carbopack A; oven temperature: 80°C; carrier gas: N_2, 20 ml/min; internal standard: 3-pentanone). With this analytical procedure, 5 ng of acetone could be reliably detected.

RESULTS

Sixty-four wipe samples of the hood used for skin painting yielded from 0 to 0·89 μg anthracene; the median was 0·02 μg. Wipe samples of the equipment used in skin painting (containers for anthracene solution, acetone, and wastes, pipetter, pipetter tips and rack, scissors, transfer cage) usually yielded anthracene. As expected, the disposable pipette tips were most heavily contaminated with anthracene; 36 samples ranged from 0·06 to 5·86 μg.

The data obtained from wipe samples of the worker and his garments are summarized in Table 1. The 25 data sets include eight during which skin painting was performed. The contamination obtained in skin painting could not be distinguished from that when skin painting was not performed. The worker's hands were usually contaminated although he wore gloves. Even though only small amounts of anthracene were

Table 1. *Anthracene contamination on the worker and his garments in skin-painting tests with mice*

Item sampled	Anthracene recovered (μg) Range*	Median
Jumpsuit	0·06–1·78	0·89
Gloves	0·48–1·95	1·13
Hat	0	0
Shoe covers	0–1·15	0·17
Shoes	0–0·30	0
Face	0–0·15	0·01
Hands	0–0·36	0·06

*For 25 samples.

recovered, this finding illustrates the desirability of showering after work.

The data obtained from samples of animals, and equipment used in their care and maintenance are summarized in Table 2. Nearly all the samples associated with treated animals yielded anthracene. The only samples which yielded anthracene from control animals were wipes of water bottles and cage exteriors. A single rack was used for cages containing control and treated animals. The results of the floor-wipe samples are shown in Fig. 1.

The mouse-wipe data which appear in Table 2 were obtained almost immediately after the animals were painted. To determine how long the anthracene remained on the animals, ten treated animals were sacrificed 24, 72 and 120 hr after the last skin-painting operation and wiped. No anthracene was recovered from the clipped area of these animals.

No anthracene of respirable size was collected during skin-painting operations. Of the 16 samples obtained from the stationary air samplers, seven yielded anthracene. Concentrations ranged from 3 to 66 ng/m^3. Gas-chromatographic analysis of the seven charcoal tubes for acetone vapour adsorbed during skin painting yielded acetone concentrations of from <0·1 to 0·8 ppm.

The stationary air sampler in the animal housing room yielded anthracene on 10 of 23 occasions. Concentrations ranged from 3 to 52 ng/m^3. Of the 18 samples obtained using a personal air sampler in the animal housing room during animal care and maintenance operations, seven yielded anthracene of respirable particle size. Concentrations ranged from 0·4 to 65 μg/m^3.

Table 2. *Anthracene contamination on the animals and equipment used in skin-painting tests with anthracene*

Item sampled	No. of samples	Anthracene recovered (μg) Control mice*	Treated mice*
Mouse wipes	18	0 (0)	0·25 (0·14–0·45)
Mouse hair	18	0 (0)	0·17 (0–1·39)
Mouse faeces	15	0 (0)	0·50 (0–2·54)
Bedding	17	0 (0)	4·78 (1·58–7·19)
Cage wipes	27	0 (0–0·13)	0·18 (0·06–0·63)
Water-bottle wipes	21	0·04 (0–0·10)	0·16 (0·08–0·30)
Rack wipes	21	0·20 (0–0·82)†	

*Median (range).
†One rack was used for control and treated animals.

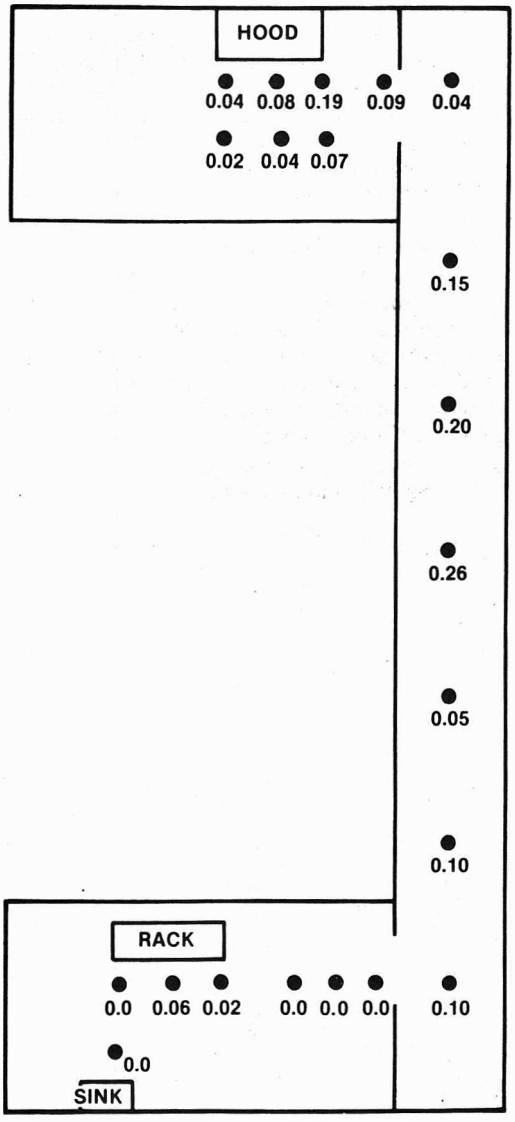

Fig. 1. Floor sampling locations and median amounts of anthracene (μg) recovered from the skin-painting room (top), hallway and animal-housing room (bottom).

DISCUSSION

In general, the magnitudes of worker and environmental contamination found in this study were very low. After skin-painting operations the jumpsuit and gloves yielded less than 2 μg anthracene, no respirable anthracene was detected, and the average concentration of acetone to which the worker was exposed was less than 1 ppm. Following animal care and maintenance procedures the jumpsuit and gloves yielded less than 2 μg anthracene, and concentrations of respirable anthracene were less than 65 μg/m^3. The highest median floor concentration was 0·26 μg anthracene; the highest floor wipe was less than 6 μg. No anthracene was recovered from the animals' dorsal area 24 hr after skin painting.

These findings indicate that the hood retains nearly all the anthracene and acetone used to paint the 175 animals (about 7·5 mg and 8·75 ml, respectively). The

respirable anthracene found in the animal housing room probably had been excreted by the animals and re-entrained during cage and bedding changing. Floor contamination was probably due to the anthracene which escaped from the hood in skin painting and that which was re-entrained in cage and bedding changing. The contamination was probably disseminated during movement of the rack and waste materials. Contamination could probably be reduced still further were wire grids substituted for bedding.

We think that the low levels of contamination observed in this study, in contrast to the rather high levels reported previously (Darlow *et al.* 1969), resulted from skin painting within a ventilated enclosure and using a solute that did not remain on the skin surface for extended periods of time. We conclude that the procedures described provide adequate safeguards for personnel and the environment when skin painting with a non-volatile solute and a solvent of low toxicity. Similar procedures would probably be adequate for administration of test chemicals by gavage.

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REFERENCES

AIHA Aerosol Technology Committee (1970). A guide for respirable mass sampling. *Am. ind. Hyg. Ass. J.* **31**, 133.

Darlow, H. M., Simmons, D. J. C. & Roe, F. J. C. (1969). Hazards from experimental skin painting of carcinogens. *Archs. Envir. Hlth* **18**, 883.

National Cancer Institute USDHEW (1968–1977). *Survey of Compounds which have been Tested for Carcinogenic Activity, Volumes 1–7.* U.S. Government Printing Office, Washington, D.C.

Ed Cosmet. Toxicol. Vol. 17, pp. 353 to 355

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SHORT PAPER

TOXICITY OF NOVEL SESQUITERPENOIDS FROM THE STRESSED SWEET POTATO (*IPOMOEA BATATAS*)

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Abstract—Five three-substituted furans, recently isolated from stressed sweet potato root tissue, were injected into mice to obtain LD_{50} values and to study the gross and microscopic pathology. All compounds produced temporary neurological effects followed by development of extensive necrosis in the liver. The LD_{50}s varied from 184 to 266 mg/kg except for the most toxic compound, 6-myoporol, which had an LD_{50} of 84 ± 10 mg/kg. The former values are comparable to those for ipomeamarone, usually the most abundant sesquiterpenoid found in stressed sweet potato tissue.

Introduction

Sweet potato root tissue subjected to various stress factors responds by synthesizing several substances toxic to various animal species. Currently of most interest are the three-substituted furans which may be grouped into two major categories based primarily on their toxicity to the liver or lungs.

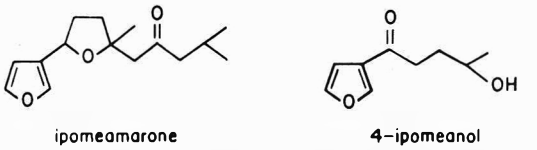
The most widely studied example of the hepatotoxic group is ipomeamarone (below), a sesquiterpenoid whose formation in the sweet potato is stimulated by several agents including contact with mercuric chloride, infection by the black-rot fungus, *Ceratocystis fimbriata*, and by Fusarium-rot fungi (Burka & Wilson, 1976). Ipomeamarone (Fig. 1) is usually the most abundant three-substituted furan and is almost invariably found in stressed sweet potato tissue.

The pulmonary toxins are somewhat simpler C_9 compounds whose origin has been attributed solely to infection by certain species of Fusarium (Boyd, Burka, Harris & Wilson, 1974). In addition to serving as the initial stimulatory factor the fungus further acts metabolically in converting at least one of the hepatotoxins, 4-hydroxymyoporone, to pulmonary toxins (Burka, Kuhnert & Wilson, 1974). The most abundant of the latter group found in Fusarium-infected roots is 4-ipomeanol (Fig. 1). These compounds can also cause tubular necrosis in kidneys of mice surviving the respiratory disease (Boyd *et al.* 1974).

Evidence has been presented that both groups of three-substituted furans exert their toxicity after metabolic activation and covalent binding to cellular macromolecules (Boyd, Burka & Wilson, 1975; Seawright, Lee, Allen & Hrdlicka, 1978).

This report describes the toxic properties of five three-substituted furans isolated from sweet potato root tissue stressed either by application of 1% mercuric chloride or by infection with *C. fimbriata*. Methods of bioproduction, isolation and structural identification have appeared in earlier reports (Burka, Bowen, Wilson & Harris, 1974; Burka, Kuhnert, Wilson & Harris, 1977). The five compounds have been given trivial names: ipomeamaronol (Yang, Wilson & Harris, 1971), 6-myoporol (Burka & Iles, 1979), 4-hydroxymyoporone (Burka *et al.* 1977), 7-hydroxymyoporone (Burka *et al.* 1974), and dihydro-7-hydroxymyoporone (Burka, 1978). Their structures are given in Fig. 2.

Structural relationships of these compounds to normal metabolites of *Myoporum* and *Eremophila* species, shrubs found in Australia, and the Ngaio tree (*Myoporum laetum*) of New Zealand, accounts for the assigned names for three of the compounds. Myoporone, another hepatotoxic substance from these plants, has also been found in stressed sweet potato root tissue (Burka & Iles, 1979).



ipomeamarone 4-ipomeanol

Fig. 1. Structures of ipomeamarone and 4-ipomeanol.

Experimental

For toxicity studies measured doses of the respective oils were dissolved in 0·05 ml dimethylsulphoxide and injected ip into male Notre Dame strain white mice (20–23 g) obtained from Harlan Animal Industries, Inc., Indianapolis, IN. The animals were observed closely from the first few hours following injection and at frequent intervals until death or a maximum of 6 days. Animals surviving this period had recovered from any early ill effects of toxin.

For LD_{50} measurements groups of 10 to 20 mice were given one of at least three doses of a compound varying by 0·3 $\log_{10}$ intervals. The number of deaths were plotted on probit chart paper to estimate the lethal dose values (Balazs, 1970).

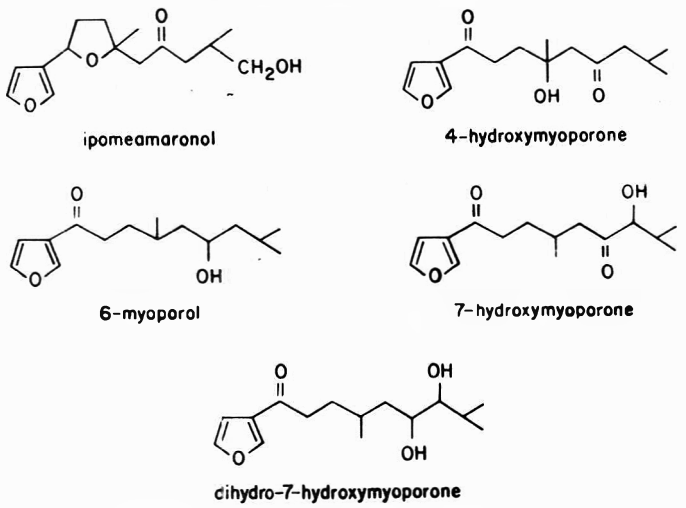


Fig. 2. Structures of recently isolated three-substituted furans from stressed sweet potato root tissue.

Additional animals were used for gross and microscopic pathology studies. Moribund individuals were killed by injecting 0·15 ml sodium pentobarbital solution (50 mg/ml) ip. Others were examined immediately after death. Sections of visceral organs, along with lungs inflated to normal volume with fixative, were submerged in 10% buffered formalin, blocked in paraffin, and stained with haematoxylin and eosin for microscopic study.

Results and Discussion

All five compounds caused marked neurological effects in mice which became evident shortly after injection. These varied with dose including ataxia, sternal recumbency, gross tremors, body rolling, and loss of righting reflex. These reactions usually disappeared within an hour and were followed by the gradual development of a semi-comatose state prior to death which usually occurred in less than 24 hr, and not more than 48 hr, after toxin injection.

Post mortem examination of animals dying in less than 24 hr revealed an enlarged and congested liver. Often lobulation was evident, and the organ was more friable than normal when handled. Animals that died 20 or more hours after injection had pale livers, and frequently there was enteritis with occult blood accumulation in the lumen of the small intestine. Other organs were unremarkable except for slight congestion of the adrenal glands. Ascitic fluid was also seen on occasion, but pleural effusion was absent.

Microscopically liver damage consisted of extensive necrosis of hepatocytes extending from the centrilobular zone nearly to the periportal zone with congested blood vessels and sinusoids. In many cases only a few cords of intact hepatocytes remained adjacent to the portal vein which contained various size globules representing fatty metamorphosis. Enteritis was seen microscopically as erosion of the villar epithelium and extensive haemorrhage filling the lumen. No evidence of pulmonary oedema was observed nor was there damage to the kidneys.

The LD_{50} ($\pm$SD) estimates, expressed as mg/kg body weight (and nmol/kg body weight), were as follows: ipomeamaronol, 266 ± 20 ($1{\cdot}00 \pm 0{\cdot}08$); 6-myoporol, 84 ± 10 ($0{\cdot}33 \pm 0{\cdot}04$); 4-hydroxymyoporone, 235 ± 85 ($0{\cdot}88 \pm 0{\cdot}32$); 7-hydroxymyoporone, 200 ± 19 ($0{\cdot}75 \pm 0{\cdot}07$); and dihydro-7-hydroxymyoporone, 184 ± 20 ($0{\cdot}69 \pm 0{\cdot}07$). These figures, with the exception of those for 6-myoporol, the most potent hepatotoxin isolated, are quite comparable to LD_{50} values for ipomeamarone, the most abundant three-substituted furan found in stressed sweet potato tissue.

Although no reports of liver disease in animals have been attributed to sweet potato consumption, it is evident that these metabolites must be considered as potentially hazardous food poisons.

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REFERENCES

Balazs, T. (1970). Measurement of acute toxicity. In *Methods in Toxicology*. Edited by G. E. Paget, p. 49. Blackwell Scientific Publications, Oxford.

Boyd, M. R., Burka, L. T., Harris, T. M. & Wilson, B. J. (1974). Lung-toxic furanoterpenoids produced by sweet potatoes (*Ipomoea batatas*) following microbial infection. *Biochim. biophys. acta.* **337**, 184.

Boyd, M. R., Burka, L. T. & Wilson, B. J. (1975). Distribution, excretion and binding of radioactivity in the rat after intraperitoneal administration of the lung-toxic furan, [^{14}C] 4-ipomeanol. *Toxic. appl. Pharmac.* **32**, 147.

Burka, L. T. (1978). 1-(3'-Furyl)-6,7-dihydroxy 4,8-dimethylnonan-1-one, a stress metabolite from sweet potatoes (*Ipomoea batatas*). *Phytochemistry* **17**, 317.

Burka, L. T., Bowen, R. M., Wilson, B. J. & Harris, T. M. (1974). 7-Hydroxymyoporone, a new toxic furanosesquiterpene from mold-damaged sweet potatoes. *J. Org. Chem.* **39**, 3241.

Burka, L. T. & Iles, J. (1979). Myoporone and related keto alcohols from stressed sweet potatoes. *Phytochemistry* **18**, 873.

Burka, L. T., Kuhnert, L. & Wilson, B. J. (1974). 4-Hydroxymyoporone, a key intermediate in the biosynthesis of pulmonary toxins produced by *Fusarium solani* infected sweet potatoes. *Tetrahedron Lett.* p. 4017.

Burka, L. T., Kuhnert, L., Wilson, B. J. & Harris, T. M. (1977). Biogenesis of lung-toxic furans produced during microbial infection of sweet potatoes (*Ipomoea batatas*). *J. Am. Chem. Soc.* **99**, 2302.

Burka, L. T. & Wilson, B. J. (1976). Toxic furanosesquiterpenoids from mold-damaged sweet potatoes (*Ipomoea batatas*). In *Mycotoxins and Other Fungal Related Food Problems*. Edited by J. Rodricks, p. 387, Advances in Chemistry, Series 149, American Chemical Society, Washington, DC .

Seawright, A. A., Lea, J. S., Allen, J. G. & Hrdlicka, J. (1978). Toxicity of *Myoporum* spp. and their furanosesquiterpenoid essential oils. In *Effects of Poisonous Plants on Livestock*. Edited by R. C. Keeler, D. R. van Kampen & L. E. James, Academic Press, N.Y.

Yang, D. T. C., Wilson, B. J. & Harris, T. M. (1971). The structure of ipomeamaronol: a new toxic furanosesquiterpene from moldy sweet potatoes. *Phytochemistry* **10**, 1653.

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MONOGRAPHS

Monographs on Fragrance Raw Materials*

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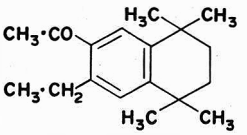
AETT

Synonyms: Acetylethyltetramethyltetralin; 6-acetyl-1,1,4,4-tetramethyl-7-ethyl-1,2,3,4-tetralin; 7-acetyl-6-ethyl-1,1,4,4-tetramethyl-1.2,3,4-tetrahydronaphthalene.
Structure:



Description and physical properties: Givaudan Index (1961).
Occurrence: Has apparently not been reported to occur in nature.
Preparation: From ethylbenzene and 2,5-dichloro-2,5-dimethylhexane (Carpenter, Easter & Wood, 1959).
Uses: In public use since the 1950s. Use in the USA amounted to approximately 100,000 lb/yr, before being voluntarily discontinued by the fragrance industry in 1977.

Concentration in final product (%):

	Soap	*Detergent*	*Creams, lotions*	*Perfume*
Usual	0·02	0·002	0·01	0·3
Maximum	0·2	0·02	0·05	1·2

Analytical data: Gas chromatogram, RIFM nos 70–70, 73–38; infra-red curve, RIFM nos 70–70, 73–38.

Status

The Council of Europe (1974) included AETT in the list of artificial flavouring substances that may be added temporarily to foodstuffs without hazard to public health. CAS Registry No. 88–29–9.

Biological data†

Preliminary studies

During routine subacute dermal tests involving exposure of rats to a perfume oil, it was observed that their skin and internal organs developed a blue colour. The central nervous system (CNS) was among the organs exhibiting the most intense colouration. A 20% (w/v) mixture of the perfume oil in corn oil was applied to the shaved dorsal skin of rats once daily on 5 days/wk for four consecutive weeks. The dose producing the effect was 600 mg/kg/day, while no effect was observed at a level of 60 mg/kg/day. In previous studies under the same test conditions, the same doses had not produced this blue-colour phenomenon in New Zealand White rabbits. Administration by ip injection produced this blue colouration in rats within a few days, but rabbits were again insensitive at doses up to 1000 mg/kg. Subsequently, the perfume oil was fractionated and each fraction was injected ip into rats; by a process of elimination, AETT was identified as the ingredient responsible for the blue colouration. The concentration of AETT in the perfume oil was 6·3%, making the dose of AETT in the perfume oil applied topically in the initial subacute study equivalent to about 38 mg/kg/day.

This identification of AETT was followed by *in vitro* experiments, consisting of immersing whole organs of previously untreated rats in ethanolic solutions of commercial-grade AETT. These experiments showed that a faint blue colour could be imparted to the ethanol when tissue, such as

*The most recent of the previous sets of these monographs appeared in *Food and Cosmetics Toxicology*, 1979, **17**, no. 3 (pp. 241–276).

† *Acknowledgement*—Avon Products Inc., The Givaudan Corp. and the Procter & Gamble Co. most generously contributed these data for evaluation by the RIFM Expert Panel and subsequently participated in a full disclosure to the US Food and Drug Administration. While the data are offered here in summary form, more extensive publications may be forthcoming.

brain, was immersed for 24 hr in 12–25% (w/v) AETT in ethanol. Using a highly purified sample of AETT (9th recrystallization) in the same *in vitro* procedure, no blue colour developed. However, when the purified sample of AETT was injected into rats, blue tissue colouration was again observed. It appeared, therefore, that AETT might be converted *in vivo* to an 'active metabolite' capable of binding with tissues to produce the blue colour.

With the demonstration that the CNS was involved in the blue colouration, studies were performed to characterize the toxic potential and to evaluate the safety of AETT for use in topically applied cosmetics and toiletries (Foster, Minner & Gressel, 1978). A brief description of each study follows.

Acute toxicity. Given as a 10% (w/v) solution in ethanol, the LD_{50} values for AETT were determined as 316 mg/kg (oral), 126 mg/kg (ip) and 584 mg/kg (unoccluded percutaneous) in female rats. Signs of toxicity preceding death were hyperactivity followed by depression and tremors, the latter occurring progressively over a period of 2–3 days before death. Autopsy showed intense blue colouration of internal tissues. Comparison of the acute ip toxicity in five species of laboratory animal revealed decreasing sensitivity to AETT as follows: rat > mouse = hamster > guinea-pig > rabbit. The rabbit never exhibited the blue-colour phenomenon, while the first four species did (Foster *et al.* 1978). On the basis of these findings, the rat, as the most sensitive species, was used for all four subchronic studies. Percutaneous administration was chosen for these tests, as the route most closely representing the major type of human exposure.

Subchronic percutaneous study in male and female rats. This study was initiated to investigate whether or not AETT might be capable of producing cumulative damage in those organs exhibiting the blue colour, particularly the CNS, and to determine the degree of systemic absorption of AETT administered topically to rats in various vehicles simulating cosmetic preparations. The doses and concentrations of AETT in four types of vehicle were as follows: (1) 1·0% (w/v) in an ethanolic vehicle at dose levels of 3 and 18 mg/kg and 0·01% (w/v) at 0·6 mg/kg; (2) 0·95% (w/v) in a waxy-base vehicle at dose levels of 1·8 and 17·1 mg/kg; (3) 0·45% (w/v) in a creamy vehicle at dose levels of 0·9 and 9·1 mg/kg; (4) 0·03% (w/v) in a liquid cream vehicle at dose levels of 0·2 and 2·2 mg/kg. The control group received no treatment. Group 1 (exposed to AETT in the ethanolic vehicle) consisted of male and female rats, while the other test groups were composed of females only.

Each test material was applied once daily by gentle inunction to an area of shaved dorsal skin equivalent to 10–20% of the total body area, on 5 days/wk for up to 26 consecutive weeks. Animals were observed daily for clinical signs of toxicity, body weight was recorded weekly, blood chemistry and haematology parameters were evaluated during wk 5, 9, 13 and 26, urine analysis was performed during wk 6, 12 and 25 and interim autopsies were performed after exposure for 13 and 26 wk. In addition, some rats given 1·0% AETT in ethanol at either 3 or 18 mg/kg were autopsied, along with controls, 4 and 12 wk after cessation of treatment. No effects on survival or body-weight gain were noted. However, varying degrees of change in appearance (blue skin) and behaviour (hyperexcitability) were noted at all dose levels. The intensity of the blue skin colour and the onset, duration, and frequency of the clinical sign of hyperexcitability were most pronounced at AETT dose levels of 9·1, 17·1 and 18 mg/kg. No compound-related effects were detected in the haematology, blood chemistry or urine analysis, with the exception of a bluish colour in the urine at the 18 mg AETT/kg level.

At autopsy, the typical blue colouration of internal organs, including the CNS, was noted at wk 13 and 26 and at wk 4 and 12 of the recovery phase. The treatments leading to blue colouration of the brain and spinal cord included 3 and 18 mg AETT/kg in ethanol, 1·8 and 17·1 mg/kg in the waxy base, 9·1 mg/kg in the creamy base and 2·2 mg/kg in the liquid cream vehicle. No blue colour was noted after exposure to 0·9 mg AETT/kg or less for 26 wk. With 9·1, 17·1 and 18 mg AETT/kg, nearly 100% of the animals exhibited blue tissue colour. At lower doses, blue tissue was observed in only a few animals (approximately 10–25%). In general, female rats were more sensitive to AETT effects than were males.

Tissues taken at autopsy at wk 13 were fixed in AFA fixative (alcohol–formalin–acetic acid), but at all other autopsies 10% neutral buffered formalin fixative was used. Histopathological evaluation of tissues taken from animals at wk 13 were unremarkable except for widespread vacuolization of the brain and spinal cord in all animals, including controls. Because it was suspected that the vacuolization was an artefact produced by the AFA fixative, the change to formalin was made. At wk 26, focal vacuolization of the brain and spinal cord was seen in rats exposed topically to 17·1 or 18 mg AETT/kg but not in controls or animals receiving lower doses of AETT. At wk 4 and 12 of the recovery period, focal vacuolization of the brain was still apparent at 18 mg AETT/kg, but again not among controls or at 3 mg/kg. As a result of these findings and to solve the question raised about possible fixative-produced vacuoles in brain tissue, a 14-wk dose-range study was initiated (Foster *et al.* 1978).

Subchronic 14-wk dose-range percutaneous toxicity study. Since female rats were found to be more sensitive to the effects of AETT than males, this study and all subsequent research was restricted to female rats. In this experiment, groups of 20 animals were exposed topically to a 10% (w/v) ethanolic solution of AETT at dose levels of 50, 100, 200 and 400 mg/kg/day. The 20 control rats were treated with ethanol. Exposure, once daily on 7 days/wk for up to 14 wk, was followed by a recovery phase of almost 20 wk after termination of treatment. Autopsy was performed on all

animals in a moribund condition and on animals selected at intervals of 2, 4, 6, 9, 11 and 14 wk during treatment and 20 wk after cessation of treatment.

Cumulative signs of toxicity consisted of hyperexcitability, motor incoordination, blue skin, hunched back, weight loss or depression, tremors and wobbly gait. All animals exposed to 200 or 400 mg AETT/kg were killed in a moribund condition within 14 and 3 days, respectively, after initiation of treatment. Except for two animals killed when moribund after 7 and 30 days of treatment with 100 mg AETT/kg, animals on 50 or 100 mg/kg survived but showed serious impairment of motor function.

At each autopsy interval, including that after the 20-wk recovery period, internal organs showed blue colouration in all AETT-treated animals. For most animals, half of the brain was fixed in 10% neutral buffered formalin and the other half in AFA fixative. Exceptions were a few animals from the control, 50 and 100 mg AETT/kg groups that were anaesthetized at wk 11 of treatment or at wk 20 of recovery and perfused *in situ* through the heart with 4% paraformaldehyde followed by 5% buffered glutaraldehyde solution. Brain, spinal cord and peripheral nerves were then stained with osmium tetroxide, embedded in epoxy resin, sectioned and stained with toluidine blue. The formalin- or AFA-fixed tissues were subjected to paraffin sectioning and stained with haematoxylin and eosin, Luxol fast blue or Bodian stain.

Histopathological examination of AFA-fixed brains again revealed widespread vacuolization of both control and AETT-treated animals. However, 10% formalin-fixed brains of control animals were normal while all AETT-treated animals exhibited dose- and time-dependent vacuolization of the brain and spinal cord as well as focal swelling and demyelinization of nerve tracts. The severity of changes varied directly with the dose and length of exposure to AETT. Vacuolization seen with AFA fixation was considered partially artefactual, masking the real effect of AETT.

The most definitive and confirmative evidence for the neuropathological potential of AETT in rats was obtained from the animals perfused *in situ* and subjected to the more elaborate preparatory technique. This technique completely ruled out artefacts and revealed two striking changes, a scattered, intra-myelinic oedema, producing demyelination visualized as a vacuolar or spongy degeneration of the white matter, and excessive pigmentation in the paranuclear cytoplasm of certain nerve cells and of central and peripheral myelinating cells. Animals examined after recovery for 20 wk exhibited the same neuropathological changes, although some degree of reversibility was noted.

RIFM's Expert Panel was informed of these findings (Foster *et al.* 1978) and subsequently a decision was made by RIFM to notify the FDA and to advise the fragrance industry that AETT should not be used.

Further subchronic percutaneous toxicity studies. AETT was used as a positive control in experiments conducted to evaluate the safety of substances of similar chemical structure and use. A 13-wk study used AETT dose levels of 0·1, 0·3, 1·0, 3·0, 10, 30 and 100 mg/kg and a second study of 26 wk duration utilized AETT dose levels of 9, 18 and 36 mg/kg. In each investigation, AETT was administered topically at varying concentrations in ethanol, and both untreated and ethanol-treated controls were used. Classical signs of neurotoxicity and neuropathological changes were detected down to and including the 9-mg/kg dose level. The no-effect level in rats was determined as 3 mg/kg during the 13-wk study, correlating with the initial 26-wk study in which the 3-mg/kg dose level was without any neuropathological effect (Foster *et al.* 1978).

Teratology study. Pregnant albino rats were exposed topically once daily on days 6–16 of gestation to either 5 or 30 mg AETT/kg dissolved at 1% (w/v) in ethanol. Foetuses were delivered on day 20 of gestation by Caesarian section and examined grossly for any abnormalities. Two thirds of the foetuses were examined for skeletal defects after Alizarin Red staining and the rest were examined by the Wilson procedure for internal organ integrity. The only effect noted was blue colouration of all maternal tissues. Foetuses were not affected, nor were any embryotoxic or teratogenic effects noted at the dose levels administered (Foster *et al.* 1978).

Mutagenicity studies. Both AETT and an *o*-diacetyl derivative (*o*-diacetyltetramethyltetralin; ODTT) were evaluated in five tester strains of Salmonella using the Ames technique. Both activation and non-activation steps of the Ames procedure were utilized. The compounds were considered not to be mutagenic under the conditions of the test (Foster *et al.* 1978).

Special studies with ODTT. Comparison of both *in vitro* and *in vivo* test results with a highly purified sample of AETT and with a commercial grade of AETT, promoted the hypothesis that AETT might be converted *in vivo* to an 'active metabolite' capable of producing the blue colour as well as the neurological changes. One such possibility, ODTT, was synthesized and given initially to mice and rats by ip injection. The results confirmed that, like AETT, ODTT was capable of producing the blue colouration of brain and spinal tissue. When given by sc injection, ODTT produced a blue colour at the injection site whereas AETT did not. In contrast to the faint blue colouration of the ethanol when a rat brain was soaked for 24 hr in 12–25% AETT, a 0·1% (w/v) ethanolic solution of ODTT turned dark blue when a rat brain was immersed in it for less than 1 hr. With rabbit brain, ODTT was also positive.

The effect of ODTT was investigated next in the rabbit, which had been shown to be insensitive to both the blue-colouring capacity and accompanying toxicity of AETT demonstrated in the rat. When injected ip into rabbits at doses up to 1000 mg/kg, ODTT failed to produce any blue colour-

ation. However, when only 15 mg ODTT/kg was administered to a rabbit by the iv route, the animal exhibited flaccid paralysis of the hind limbs and entire left side within 10 hr of the injection. Autopsy 24 hr later revealed the classic blue colour of the brain, spinal cord and peripheral nerves as well as other internal organs. Further work is planned to evaluate the possibility that the rabbit may possess the liver enzymes necessary to detoxify AETT or a metabolite such as ODTT (Foster *et al.* 1978; Manowitz, 1977).

Comparative oral study between monkeys and rats. A rhesus monkey given a daily oral dose of 50 mg AETT/kg for 16 days showed no discolouration of the brain, spinal cord or peripheral nerves (Manowitz, 1978). In contrast, rats given a daily oral dose of 50 mg/kg for 5 days showed the typical blue discolouration in the brain, spinal cord and musculature (Pennisi, Minner & Foster, 1978).

Subchronic toxicity (oral route). In three separate, 13-wk studies of detergents containing AETT as a minor ingredient, rats were fed AETT at dietary levels of 0·03, 0·05, 0·10, 0·26, 0·38, 0·52 and 1·04 ppm. There were no deaths or physical or behavioural signs of toxicity, nor were there any distinct effects on haematological or blood-chemistry values, organ weights, or organ/body weight ratios. In addition there were no gross or microscopic pathological changes related to AETT treatment and, in particular, no tissue discolouration or vacuolization of the brain was observed. Slight suppression of growth in one study and a slight increase in liver/body weight ratio in another were not correlated with AETT levels (Griffith, 1978).

Radioactivity studies. [^{14}C]AETT administered iv or ip to rats distributed rapidly through the tissues, including the CNS. Thereafter, there was a redistribution phase correlating with the movement of radiolabelled equivalents from the tissues, primarily via the liver into the gastro-intestinal tract. The faecal excretion of total equivalents was comparatively slow, indicating that there occurred an extensive reabsorption of parent compound *per se* or its metabolites to establish an enterohepatic cycle of total radiolabelled equivalents. Major portions of injected doses could not be accounted for in expired air, urine and faeces (the major pathway).

Whole-body radioautography of iv-injected rats showed a wide distribution of AETT with concentrations in lipid-rich arcs. Intestinal contents were the most dense. Liver, biliary channels and hepatic vasculature suggested an enterohepatic cycle. In the kidneys, the cortex was more dense than the medulla. Heterogeneous distribution of AETT was seen in the cerebrum, cerebellum, pons, medulla, fifth cranial nerve and spinal cord (Yesair, Liss, Kelly & Branfman, 1977).

Irritation. Applied full strength to intact or abraded rabbit skin for 24 hr under occlusion, AETT was slightly irritating (Moreno, 1973). Tested at 4% in petrolatum, it produced no irritation after a 48-hr closed-patch test on human subjects (Epstein, 1973).

Sensitization. A maximization test (Kligman, 1966; Kligman & Epstein, 1975) was carried out on 23 volunteers. The material (RIFM no. 73–38) was tested at a concentration of 4% in petrolatum and produced no sensitization reactions (Epstein, 1973).

References

Carpenter, M. S., Easter, W. M., Jr & Wood, T. F. (1959). Substituted tetrahydronaphthalenes U.S. Patent 2,897,237. 28 July 1959.

Council of Europe (1974). Natural Flavouring Substances, Their Sources, and Added Artificial Flavouring Substances. Partial Agreement in the Social and Public Health Field. List 2, no. 2220, p. 328. Strasbourg.

Epstein, W. L. (1973). Report to RIFM, 1 October.

Foster, G., Minner, R. & Gressel, Y. (1978). Personal communication (from Avon Products, Inc.) to RIFM, 7 September.

Givaudan Index (1961). *Specifications of Synthetics and Isolates for Perfumery*. 2nd Ed. p. 344. Givaudan-Delawanna, Inc., New York.

Griffith, J. F. (1978). Personal communication (from Procter & Gamble Co.) to RIFM, 15 March.

Kligman, A. M. (1966). The identification of contact allergens by human assay. III. The maximization test. A procedure for screening and rating contact sensitizers. *J. invest. Derm.* **47**, 393.

Kligman, A. M. & Epstein, W. (1975). Updating the maximization test for identifying contact allergens. *Contact Dermatitis* **1**, 231.

Manowitz, M. (1977, 1978). Personal communications (from Givaudan Corp.) to RIFM.

Moreno, O. M. (1973). Report to RIFM, 21 September.

Pennisi, S., Minner, R. & Foster, G. (1978). Personal communication (from Avon Products, Inc.) to RIFM, 18 September.

Yesair, D. W., Liss, R. H., Kelly, C. J. & Branfman, A. R. (1977). Physiological disposition of ^{14}C-Giv 82-2028 in rats. Arthur D. Little Report to the Givaudan Corporation, 17 October.

Additional References

Spencer, P. S., Sterman, A. B., Horoupian, D., Bischoff, M. & Foster, G. (1979). Neurotoxic changes in rats exposed to the fragrance compound acetyl ethyl tetramethyl tetralin. *Neurotoxicology* **1** (1), 221.

Spencer, P. S., Sterman, A. B., Horoupian, D. S. & Foulds, M. M. (1979). Neurotoxic fragrance produces ceroid and myelin disease. *Science, N.Y.* **204**, 633.

DIMETHYL CARBONATE

Synonym: Methyl carbonate.
Structure: $CH_3 \cdot O \cdot CO \cdot O \cdot CH_3$.
Description and physical properties: *Merck Index* (1976).
Occurrence: Has apparently not been reported to occur in nature.
Preparation: From methyl chloroformate by reaction with methanal.
Uses: Use in fragrances in the USA amounts to less than 1000 lb/yr.
Concentration in final product (%):

	Soap	*Detergent*	*Creams, lotions*	*Perfume*
Usual	0·005	0·003	0·003	0·08
Maximum	0·1	0·01	0·02	0·4

Analytical data: Gas chromatogram, RIFM no. 76–82; infra-red curve, RIFM no. 76–82.

Status

Dimethyl carbonate is not included in the listings of the FDA, FEMA (1965) or the Council of Europe (1974), or in the *Food Chemicals Codex* (1972). CAS Registry No. 616–38–6.

Biological data

Acute toxicity. In mice and rats, the acute oral LD_{50} has been reported as 6·4–12·8 g/kg and the ip LD_{50} as 800–1600 mg/kg (Fassett, 1963). The dermal LD_{50} in guinea-pigs was reported as > 10 ml/kg (Fassett, 1963), while that in rabbits exceeded 5 g/kg (Levenstein, 1976).

Inhalation. Inhalation of dimethyl carbonate vapour at a concentration of 8000 ppm produced in the rat a rapid onset of gasping, loss of co-ordination, frothing from the mouth and nose, and pulmonary oedema, with death ensuing within 2 hr (Fassett, 1963). However, Torkelson, Kary, Chenoweth & Larsen (1971) observed very little effect in rats inhaling dimethyl carbonate, even after 7-hr exposures at calculated concentrations of 10,000 ppm (analysis > 8700 ppm). No response was noted during exposure, and pathological changes were inconsistent in those animals subsequently examined. The difference in response recorded in these two studies suggests that the earlier work may have been done with an impure sample. Gage (1970) reported that rats exposed to 5000 ppm for 6 hr displayed eye irritation, salivation, respiratory difficulty and incoordination, but did not die, and they recovered rapidly after exposure. Autopsy showed no abnormalities in the internal organs. Rats subjected to fifteen 6-hr exposures to a vapour concentration of 1000 ppm showed no toxic signs; autopsy revealed no abnormalities of the internal organs (Gage, 1970).

Irritation. Dimethyl carbonate applied full strength to intact or abraded rabbit skin for 24 hr under occlusion was not irritating (Levenstein, 1976). Tested at 4% in petrolatum, it produced no irritation after a 48-hr closed-patch test on human subjects (Kligman, 1976). It is claimed that a mosquito- and wood-lice-repellent preparation, 15% of which consisted of a mixture of dimethyl phthalate, indalone and dimethyl carbonate, had no adverse effect when applied to human skin (Petrishcheva, Saf'yanova, Budak & Gaiko, 1956).

Sensitization. A maximization test (Kligman, 1966; Kligman & Epstein, 1975) was carried out on 25 volunteers. The material (RIFM no. 76–82) was tested at a concentration of 4% in petrolatum and produced no sensitization reactions (Kligman, 1976).

Metabolism. It has been suggested that dimethyl carbonate acts as a methylating agent in tissues (Fassett, 1963).

References

Council of Europe (1974). Natural Flavouring Substances, Their Sources, and Added Artificial Flavouring Substances. Partial Agreement in the Social and Public Health Field. Strasbourg.

Fassett, D. W. (1963). Esters. In *Industrial Hygiene and Toxicology*. 2nd Ed. Edited by F. A. Patty. Vol. II, p. 1913. Interscience Publishers, New York.

Flavoring Extract Manufacturers' Association (1965). Survey of flavoring ingredient usage levels. *Fd Technol., Champaign* **19**(2), part 2, 155.

Food Chemicals Codex (1972). 2nd Ed. Prepared by the Committee on Specifications, Food Chemicals Codex, of the Committee on Food Protection. National Academy of Sciences–National Research Council Publ. 1406, Washington, DC.

Gage, J. C. (1970). The subacute inhalation toxicity of 109 industrial chemicals. *Br. J. ind. Med.* **27**, 1.

Kligman, A. M. (1966). The identification of contact allergens by human assay. III. The maximization test. A procedure for screening and rating contact sensitizers. *J. invest. Derm.* **47**, 393.

Kligman, A. M. (1976). Report to RIFM, 20 April.

Kligman, A. M. & Epstein, W. (1975). Updating the maximization test for identifying contact allergens. *Contact Dermatitis* **1**, 231.

Levenstein, I. (1976). Report to RIFM, 18 May.

Merck Index (1976). *An Encyclopedia of Chemicals and Drugs*. 9th Ed. No. 5912. Merck & Co., Inc., Rahway, NJ.

Petrishcheva, P. A., Saf'yanova, V. M., Budak, A. P. & Gaiko, B. A. (1956) New mosquito repellents from the Scientific Research Institute of Fertilizers and Insectifuges. *Voenno-med. Zh., Leningr.* no. 7, p. 49.

Torkelson, T. R., Kary, C. D., Chenoweth, M. B. & Larsen, E. R. (1971). Single exposure of rats to the vapors of trace substances in methoxyflurane. *Toxic. appl. Pharmac.* **19**, 1.

Additional references

Ough, C. S. & Langbehn, L. (1976). Measurement of methylcarbamate formed by the addition of dimethyl dicarbonate to model solutions and to wines. *J. Agric. Fd Chem.* **24**, 428.

Burditt, A. K., Jr., Hinman, F. G. & Balock, J. W. (1963). Screening of fumigants for toxicity to eggs and larvae of the oriental fruit fly and Mediterranean fruit fly. *J. econ. Ent.* **56**, 261.

DIMETHYL MALONATE

Synonym: Methyl malonate.
Structure: $CH_3 \cdot OCO \cdot CH_2 \cdot OCO \cdot CH_3$.
Description and physical properties: *Merck Index* (1976).
Occurrence: Has apparently not been reported to occur in nature.
Preparation: By direct esterification of methanol with malonic acid using azeotropic conditions (Arctander, 1969).
Uses: In public use since the 1950s. Use in fragrances in the USA amounts to less than 1000 lb/yr. Concentration in final product (%):

	Soap	*Detergent*	*Creams, lotions*	*Perfume*
Usual	0·02	0·002	0·01	0·2
Maximum	0·2	0·02	0·05	0·8

Analytical data: Gas chromatogram, RIFM no. 76–85; infra-red curve, RIFM no. 76–85.

Status

The Council of Europe (1974) included dimethyl malonate in the list of artificial flavouring substances not fully evaluated. CAS Registry No. 108–59–8.

Biological data

Acute toxicity. The acute oral LD_{50} in rats was reported as 4·62 ml/kg (3·98–5·36 ml/kg) and the acute dermal LD_{50} in rabbits exceeded 5 g/kg (Levenstein, 1976).

Irritation. Dimethyl malonate applied full strength to intact or abraded rabbit skin for 24 hr under occlusion was irritating (Levenstein, 1976). Tested at 8% in petrolatum, it produced no irritation after a 48-hr closed-patch test on human subjects (Kligman, 1976).

Sensitization. A maximization test (Kligman, 1966; Kligman & Epstein, 1975) was carried out on 25 volunteers. The material (RIFM no. 76–85) was tested at a concentration of 8% in petrolatum and produced no sensitization reactions (Kligman, 1976).

Nutrition. Dimethyl malonate, constituting 5% of the diet of growing chicks, provided no available energy and rendered the feed somewhat less palatable than the control diet (Yoshida, Morimoto & Oda, 1970).

References

Arctander, S. (1969). *Perfume and Flavor Chemicals (Aroma Chemicals)*. Vol. 2, no. 2098. S. Arctander, Montclair, NJ.

Council of Europe (1974). Natural Flavouring Substances, Their Sources, and Added Artificial Flavouring Substances. Partial Agreement in the Social and Public Health Field. List 3, Section (A), no. 4080, p. 365. Strasbourg.

Kligman, A. M. (1966). The identification of contact allergens by human assay. III. The maximization test. A procedure for screening and rating contact sensitizers. *J. invest. Derm.* **47**, 393.

Kligman, A. M. (1976). Report to RIFM, 11 May.

Kligman, A. M. & Epstein, W. (1975). Updating the maximization test for identifying contact allergens. *Contact Dermatitis* **1**, 231.

Levenstein, I. (1976). Report to RIFM, 18 May.

Merck Index (1976). *An Encyclopedia of Chemicals and Drugs*. 9th Ed. No. 5961. Merck & Co., Inc., Rahway, NJ.

Yoshida, M., Morimoto, H. & Oda, R. (1970). Availability of energy in esters of aliphatic acids and alcohols by growing chicks. *Agric. biol. Chem.* **34**, 1668.

DIMETHYL SULPHIDE

Synonym: Methyl sulphide.
Structure: $CH_3 \cdot S \cdot CH_3$.
Description and physical properties: Merck Index (1976).
Occurrence: Reported to have been found in American peppermint oil, the oil of Algerian geranium and butter, and also in white bread (*Fenaroli's Handbook of Flavor Ingredients*, 1975).
Preparation: From potassium methylsulphate plus potassium sulphide (Arctander, 1969).
Uses: In public use since the 1930s. Use in fragrances in the USA amounts to approximately 1000 lb/yr.

Concentration in final product (%):

	Soap	*Detergent*	*Creams, lotions*	*Perfume*
Usual	0·001	0·0001	0·0005	0·008
Maximum	0·03	0·003	0·005	0·04

Analytical data: Gas chromatogram, RIFM no. 75–41; infra-red curve, RIFM no. 75–41.

Status

Dimethyl sulphide (DMS) was given GRAS status by FEMA (1965) and is approved by the FDA for food use (21 CFR 172.515). The Council of Europe (1974) included DMS, at a level of 1·5 ppm, in the list of artificial flavouring substances that may be added to foodstuffs without hazard to public health. CAS Registry No. 75–18–3.

Biological data

Acute toxicity. The oral LD_{50} for DMS was reported to be 535 and 3700 mg/kg in rats and mice, respectively (National Institute for Occupational Safety and Health, 1976). Koptyaev (1967) reported LD_{100} values of 5400 and 6300 mg/kg and LD_{50} values of 3300 and 3700 mg/kg for rats and mice, respectively, in each case, but the route of administration was not stated. The acute intoxication was characterized by disturbed co-ordination of movement, a deeply narcotic state and then death. Other effects of DMS were a decrease in oxygen demand, especially when the compound was administered in a dose of 660 mg/kg, marked changes in blood-catalase activity, a decrease in –SH-group content, decreases in the weight of lungs and liver, and an increase in the weight of the adrenals (Koptyaev, 1967). The acute dermal LD_{50} value in rabbits was reported as >5 g/kg (Levenstein, 1975).

The toxicity of DMS was found to be greater than that of dimethylsulphoxide (DMSO), and the signs of DMS poisoning resembled those of benzene poisoning (Filippova, 1972). DMS is highly toxic to warm-blooded animals; biochemical analysis of the changes in the rate of redox processes, the glycogen-forming function of the liver, and the level of amino acids in the blood classified this compound as a polytropic poison (Selyuzhitskii & Timofeev, 1972). These authors reported that at threshold concentration, DMS stimulated reflex changes to light sensitivity of the eye during the course of dark adaptation, and that the latent effect of DMS impaired the bioelectric activity of the respiratory muscles in warm-blooded animals.

Subacute toxicity. In groups of 15 male and 15 female rats given DMS by daily oral intubation at dose levels of 0 (control), 2·5, 25·0 and 250·0 mg/kg/day for 14 wk, there were no effects on the rate of body-weight gain, intake of food and water, results of haematological examinations, serum-enzyme levels, urinary cell excretion, renal concentration tests, organ weights or histopathological examinations that were attributable to treatment (Butterworth, Carpanini, Gaunt, Hardy, Kiss & Gangolli, 1975). Consequently the no-untoward-effect level for DMS in this study was taken as 250 mg/kg/day.

The lungs of rabbits treated for 13 wk with a 2% solution of DMS in the drinking-water (to provide an approximate intake of 2 g/kg/day) were heavier than those of the control group, and at autopsy, they appeared to have more congestion, with some haemorrhagic spots (Wood, Wirth, Weber & Palmquist, 1971). Kidneys of the DMS-treated rabbits showed gross evidence of pyelonephritis.

Inhalation. Ten groups each of 15 rats were exposed to DMS over a range of atmospheric concentrations (Selyuzhitskii, 1972). In those exposed to 25 mg/m^3 for 6 hr/day for 6 months, a decrease in weight gain was noted, together with an increase in heart weight, disorders of corticosteroid distribution in the adrenal tissue, a decrease in oxygen consumption and carbon dioxide elimination, decreases in catalase activity and the methaemoglobin and phospholipid levels in the blood, and increases in pyruvic acid, lactic acid and thiol groups in whole blood and in tissue homogenates. DMS also increased cholesterol levels in the blood serum. The lowest DMS concentration that produced temporary changes under these experimental conditions was 5 mg/m^3. In mice and rats

the LC_{16} levels were, respectively, 20·15 and 30·2 mg/m^3, the LC_{50} values were 31·62 and 50·12 mg/m^3, and the LC_{84} values were 54·95 and 91·2 mg/m^3 (Selyuzhitskii, 1972).

The possible role of mercaptans and their metabolites in hepatic coma was studied in rats by Zieve, Doizaki & Zieve (1974). The CD_{50} (coma dose) in rats was reported as 9·6% (v/v) DMS in air. The blood level of DMS at which coma occurred was 7 μmol/ml blood. In synergism studies, each rat received ip injections of 2 ml ammonium acetate or ammonium chloride solution before being placed in an inhalation chamber. DMS decreased the CD_{50} of the ammonium ion by approximately 50% and the incidence of coma rose from 0 to 100% when the maximum subcoma dose of DMS (6·36% in air) and the ammonium ion were given together.

Irritation. Upon application to two thirds of the tail of rats, DMS penetrated the skin and mucous membranes and produced epidermal hyperkeratosis, and when applied to the conjunctival sac of rabbits, DMS caused changes in the sclera (Selyuzhitskii, 1972).

Applied full strength to intact or abraded rabbit skin for 24 hr under occlusion, DMS was slightly irritating (Levenstein, 1975). Tested at 1% in petrolatum, it produced no irritation after a 48-hr closed-patch test on human subjects (Kligman, 1975).

Sensitization. A maximization test (Kligman, 1966; Kligman & Epstein, 1975) was carried out on 25 volunteers. The material (RIFM no. 75–41) was tested at a concentration of 1% in petrolatum and produced no sensitization reactions (Kligman, 1975).

Metabolism. DMS diluted with an equal volume of sesame seed oil was administered sc to rabbits on each of four consecutive days, and the urine was collected and analysed for 6 days (Williams, Burstein & Layne, 1966). After 4 days of continuous analysis of the urine, 374 mg DMSO (8·6% of the original dose of DMS) and 504 mg dimethylsulphone ($DMSO_2$; 9·8% of the DMS dose) were recovered. These results indicated that DMSO and $DMSO_2$ were the principal metabolites of DMS, and suggested that the remainder of the dose was probably expired unchanged.

When [^{3}H]DMSO was incubated with dialysed and undialysed extracts of rabbit-lens protein, [^{3}H]DMS was generated from both fractions, and from mixtures of [^{3}H]DMSO with either cysteine or glutathione (Wood *et al.* 1971). No reaction occurred with methionine or ascorbic acid.

DMS was a weak inhibitor of microsomal Na^+/K^+-stimulated ATPase from rat brain (Foster, Ahmed & Zieve, 1974).

Pharmacology. When benzene was dissolved in DMS prior to administration to rats, no increase in benzene lethality was observed (Koscis, Harkaway & Snyder, 1975). In a series of experiments, these authors found that DMS exerted no protective effect against the lethality of the anticholinesterase agents paraoxon and OMPA, while in two rats exposed to temperatures of 5°C, doses of 2·5 ml DMS/kg had a strongly hypothermic effect, decreasing the body temperature by about 18°C during the first hour and depressing it below 20°C during the second hour. The rats were difficult to revive upon re-exposure to room temperature. Both oral and ip administration of DMS to mice reduced their motor activity significantly (Koscis *et al.* 1975). Using ^{131}I-uptake tests in rats, DMS was found to inhibit thyroid function significantly (Saghir, Cowan & Salji, 1967).

Distribution in biological fluids. The concentration of DMS detected in human saliva has been decreased by the oral administration of ascorbic acid or other oral preparations (Kaizu, 1976; Larsson, 1973). DMS has been detected in the breath of patients with cirrhosis of the liver or hepatic coma who had received methionine in the diet (Chen, Zieve & Mahadevan, 1970), and also in the milk, blood and urine of cows (Bassette, Turner & Ward, 1966; Gordon & Morgan, 1972; Loney, Bassette & Ward, 1963; Reddy, Bassette, Ward & Dunham, 1967). Administration of DMSO to cattle produced detectable levels of DMS in the exhaled air (Tiews, Floegel, Scharrer & Harre, 1972; Tiews, Scharrer, Harre & Floegel, 1975). The production of DMS following DMSO administration to animals has been reviewed by Wood *et al.* (1971). DMS represented less than 5% of the administered dose of DMSO in most species (Koscis *et al.* 1975; DiStefano, 1971; Wong, Wang, Dreyfuss & Schreiber, 1971).

DMS has also been produced *in vitro* from incubation of *S*-methylcysteinesulphoxide with goat-rumen fractions (Smith, Earl & Matheson, 1974).

References

Arctander, S. (1969). *Perfume and Flavor Chemicals (Aroma Chemicals)*. Vol. 1, no. 1060. S. Arctander, Montclair, NJ.

Bassette, R., Turner, M. E. & Ward, G. (1966). Volatile compounds in blood, milk, and urine of cows fed silage-grain, bromegrass pasture, and hay-grain test meals. *J. Dairy Sci.* **49**, 811.

Butterworth, K. R., Carpanini, F. M. B., Gaunt, I. F., Hardy, J., Kiss, I. S. & Gangolli, S. D. (1975). Short-term toxicity of dimethyl sulphide in the rat. *Fd Cosmet. Toxicol.* **13**, 15.

Chen, S., Zieve, L. & Mahadevan, V. (1970). Mercaptans and dimethyl sulfide in the breath of patients with cirrhosis of the liver. Effect of feeding methionine. *J. Lab. clin. Med.* **75**, 628.

Council of Europe (1974). Natural Flavouring Substances, Their Sources, and Added Artificial Flavouring Substances. Partial Agreement in the Social and Public Health Field. List (1). no. 483, p. 225. Strasbourg.

DiStefano, V. (1971). Pharmacological properties of antiirradiation drugs. US National Technical Information Service Report COO-3492-13.

Fenaroli's Handbook of Flavor Ingredients (1975). Edited by T. E. Furia and N. Bellanca. 2nd Ed. Vol. II, pp. 406 & 699. CRC Press, Cleveland, OH.

Filippova, Z.Kh. (1972). Toxic properties of dimethyl sulfoxide and sulfide. *Khim. Seraorg. Soedin., Soderzh. Neftyakh. Nefteprod.* **9**, 562.

Flavoring Extract Manufacturers' Association (1965). Survey of flavoring ingredient usage levels. No. 2746. *Fd Technol., Champaign* **19**(2), part 2, 155.

Foster, D., Ahmed, K. & Zieve, L. (1974). Action of methanethiol on Na^+,K^+-ATPase: Implications for hepatic coma. *Ann. N.Y. Acad. Sci.* **242**, 573.

Gordon, D. T. & Morgan, M. E. (1972). Principal volatile compounds in feed flavored milk. *J. Dairy Sci.* **55**, 905.

Kaizu, T. (1976). Source of foul breath and its control. *Nippon Shika Ishikai Zasshi* **29**, 228.

Kligman, A. M. (1966). The identification of contact allergens by human assay. III. The maximization test. A procedure for screening and rating contact sensitizers. *J. invest. Derm.* **47**, 393.

Kligman, A. M. (1975). Report to RIFM, 17 June.

Kligman, A. M. & Epstein, W. (1975). Updating the maximization test for identifying contact allergens. *Contact Dermatitis* **1**, 231.

Koscis, J. J., Harkaway, S. & Snyder, R. (1975). Biological effects of the metabolites of dimethyl sulfoxide. *Ann. N.Y. Acad. Sci.* **243**, 104.

Koptyaev, V. G. (1967). Sanitary-toxicological characteristics of dimethyl sulfide. In *28th Mater. Nauch. Sess. Arkhangel'sk. Gos. Med. Inst.* Edited by N. P. Bychikhin. p. 74. Sev.-Zapad. Knizhnoe Izd., Arkhangelsk, USSR.

Larsson, B. T. (1973). Gas chromatographic study of the effect of ascorbic acid oxidation on the formation of volatiles in saliva samples. *Scand. J. dent. Res.* **81**, 22.

Levenstein, I. (1975). Report to RIFM, 10 June.

Loney, B. E., Bassette, R. & Ward, G. M. (1963). Volatile components in milk, blood, and urine from cows fed silage, bromegrass, and hay and grain. *J. Dairy Sci.* **46**, 922.

Merck Index (1976). *An Encyclopedia of Chemicals and Drugs.* 9th Ed. No. 5996. Merck & Co., Inc., Rahway, NJ.

National Institute for Occupational Safety and Health (1976). *Registry of Toxic Effects of Chemical Substances.* Edited by H. E. Christensen and E. J. Fairchild. Entry no. PV50750, p. 722. NIOSH, Washington, DC.

Reddy, M. C., Bassette, R., Ward, G. & Dunham, J. R. (1967). Relations of methyl sulfide and flavor score of milk. *J. Dairy Sci.* **50**, 147.

Saghir, A. R., Cowan, J. W. & Salji, J. P. (1967). Antithyroid activity of volatile components of Allium. Proceedings of 3rd Symposium on Human Nutrition and Health in the Near East. p. 154.

Selyuzhitskii, G. V. (1972). Experimental data used to determine the maximum permissible concentration of methyl mercaptan, dimethyl sulfide, and dimethyl disulfide in the air of the production area of paper and pulp plants. *Gig. Truda prof. Zabol.* **16**, 46.

Selyuzhitskii, G. V. & Timofeev, V. P. (1972). Sanitary-toxicological study of the sulfur-containing components from sulfate pulp manufacture emissions. *Khim. Seraorg. Soedin., Soderzh. Neftyakh. Nefteprod.* **9**, 587.

Smith, R. H., Earl, C. R. & Matheson, N. A. (1974). Probable role of S-methylcysteine sulfoxide in kale poisoning in ruminants. *Biochem. Soc. Trans.* **2**, 101.

Tiews, J., Floegel, L., Scharrer, E. & Harre, N. (1972). Excretion and metabolism of carbon-14-labeled dimethyl sulfoxide in calves after cutaneous and subcutaneous administration. *Berl. Münch. tierärztl. Wschr.* **85** (9), 161.

Tiews, J., Scharrer, E., Harre, N. & Floegel, L. (1975). Metabolism and excretion of dimethyl sulfoxide in cows and calves after topical and parenteral application. *Ann. N.Y. Acad. Sci.* **243**, 149.

Williams, K. I. H., Burstein, S. H. & Layne, D. S. (1966). Metabolism of dimethyl sulfide, dimethyl sulfoxide, and dimethyl sulfone in the rabbit. *Archs Biochem. Biophys.* **117**, 84.

Wong, K. K. K., Wang, G. M., Dreyfuss, J. & Schreiber, E. C. (1971). Absorption, excretion, and biotransformation of dimethyl sulfoxide in man and miniature pigs after topical application as an 80% gel. *J. invest. Derm.* **56**, 44.

Wood, D. C., Wirth, N. V., Weber, F. S. & Palmquist, M. A. (1971). Mechanism considerations of dimethyl sulfoxide (DMSO)-lenticular changes in rabbits. *J. Pharmac. exp. Ther.* **177**, 528.

Zieve, L. Doizaki, W. M. & Zieve, F. J. (1974). Synergism between mercaptans and ammonia or fatty acids in the production of coma: A possible role for mercaptans in the pathogenesis of hepatic coma. *J. Lab. clin. Med.* **83**, 16.

Additional references

Micro-organisms

Block, S. S., Weidner, J. P. & Walsh, A. (1970). Sulfur disinfectants: antimicrobial activity of thiolsulfonates. *Proc. Chem. Spec. Mfrs' Ass., Proc. Ann. Meet. 1969*, **56**, 117.

Dykstra, G. J., Drerup, D. L., Branen, A. L. & Keenan, T. W. (1971). Formation of dimethyl sulfide by *Propionibacterium shermanii* ATCC 9617. *J. Dairy Sci.* **54**, 168.

Freeman, L. R., Silverman, G. J., Angelini, P., Merritt, C., Jr. & Esselen, W. B. (1976). Volatiles produced by microorganisms isolated from refrigerated chicken at spoilage. *Appl. envir. Microbiol.* **32** (2), 222.

Grey, T. C. & Lea, C. H. (1969). Chemical and organoleptic changes in poultry meat resulting from the growth of psychrophylic spoilage bacteria at 1°C. VI. Volatile aroma constituents. *Br. Poult. Sci.* **10**, 303.

Katayama, T. (1964). Volatile constituents of algae. XX. Pharmacological action of volatile constituents and biochemical significance of the existence of acrylic acid. *Kagoshima Daigaku Suisan Gakubu Kiyo* **13**, 58.

Kulshrestha, D. C. & Marth, E. H. (1970). Growth and activity of *Streptococcus cremoris* in skimmilk fortified with some volatile compounds. *J. Milk Fd Technol.* **33**, 492.

Kulshrestha, D. C. & Marth, E. H. (1974). Inhibition of bacteria by some volatile and nonvolatile compounds associated with milk. I. *Escherichia coli*. III. *Staphylococcus aureus*. IV. *Streptococcus lactis*. V. *Leuconostoc citrovorum*. VI. *Streptococcus thermophilus*. *J. Milk Fd Technol.* **37**, 510, 545, 593, 600 & 606.

Rasmussen, R. A. (1974). Emission of biogenic hydrogen sulfide. *Tellus* **26** (1), 254.

Salsbury, R. L., Marvil, D. K., Woodmansee, C. W. & Haenlein, G. F. W. (1971). Utilization of methionine and methionine hydroxy analog by rumen microorganisms *in vitro*. *J. Dairy Sci.* **54**, 390.

Sivela, S. & Sundman, V. (1975). Demonstration of *Thiobacillus*-type bacteria which utilize methyl sulphides. *Archs Mikrobiol.* **103**, 303.

Toan, T. T., Bassette, R. & Claydon, T. J. (1965). Methyl sulfide production by *Aerobacter aerogenes* in milk. *J. Dairy Sci.* **48**, 1174.

Invertebrates

Katayama, T. (1964). Volatile constituents of algae. XX. Pharmacological action of volatile constituents and biochemical significance of the existence of acrylic acid. *Kagoshima Daigaku Suisan Gakubu Kiyo* **13**, 58.

Occurrence in fish tissues

Ackman, R. G., Hingley, J. & MacKay, K. T. (1972). Dimethyl sulfide as an odor component in Nova Scotia fall mackerel. *J. Fish. Res. Bd Can.* **29**, 1085.

Angelini, P., Merritt, C., Jr., Mendelsohn, J. M. & King, F. J. (1975). Effect of irradiation on volatile constituents of stored haddock flesh. *J. Fd Sci.* **40**, 197.

Miller, A., III, Scanlan, R. A., Lee, J. S. & Libbey, L. M. (1972). Volatile compounds produced in ground muscle tissue of canary rockfish (Sebastes pinniger) stored on ice. *J. Fish. Res. Bd Can.* **29**, 1125.

Miller, A., III, Scanlan, R. A., Lee, J. S. & Libbey, L. M. (1973). Identification of the volatile compounds produced in sterile fish muscle (Sebastes melanops) by *Pseudomonas fragi*. *Appl. Microbiol.* **25**, 952.

Sipos, J. C. & Ackman, R. G. (1964). Association of dimethyl sulfide with the 'blackberry' problem in cod from the Labrador area. *J. Fish. Res. Bd Can.* **21**, 423.

FIR BALSAM OREGON

Synonym: Oregon balsam.

Description and physical properties: A light amber-coloured to pale-yellow liquid. The constituents of fir balsam oregon include *l*-α- and *l*-β-pinene, *l*-limonene and *l*-α-terpineol (Guenther, 1952).

Occurrence: Found in the trunk of *Pseudotsuga taxifolia* (*Pseudotsuga menzieii*) (Poir.) Britt. (Fam. Pinaceae) (Guenther, 1952).

Preparation: By collection from felled trees or by insertion of a tube into the live tree (Guenther, 1952).

Uses: In public use since the 1930s. Use in fragrances in the USA amounts to approximately 1000 lb/yr.

Concentration in final product (%):

	Soap	*Detergent*	*Creams, lotions*	*Perfume*
Usual	0·02	0·002	0·01	0·2
Maximum	0·3	0·03	0·1	0·8

Status

Fir balsam oregon is not included in the listings of the FDA, FEMA (1965) or the Council of Europe (1974), or in the *Food Chemicals Codex* (1972).

Biological data

Acute toxicity. Both the acute oral LD_{50} in rats and the acute dermal LD_{50} in rabbits exceeded 5 g/kg (McGee, 1974).

Irritation. Fir balsam oregon applied to the backs of hairless mice was not irritating (Urbach & Forbes, 1974). Applied full strength to intact or abraded rabbit skin for 24 hr under occlusion it was not irritating (McGee, 1974). Tested at 8% in petrolatum it produced no irritation after a 48-hr closed-patch test on human subjects (Kligman, 1975).

Sensitization. A maximization test (Kligman, 1966; Kligman & Epstein, 1975) was carried out on 25 volunteers. The material (RIFM no. 74–165) was tested at a concentration of 8% in petrolatum and produced no sensitization reactions (Kligman, 1975).

Phototoxicity. No phototoxic effects were reported for undiluted fir balsam oregon on hairless mice and swine (Urbach & Forbes, 1974).

Tumour incidence. The effect of bedding materials on tumour incidence has been the subject of studies in Australia (Sabine, 1975; Sabine, Horton & Wicks, 1973) and in the USA (Heston, 1975). It appears that the low tumour incidence in C3H mice bedded in sawdust from Douglas fir (*Pseudotsuga* spp.), reported by Sabine *et al.* (1973) was a result of poor health from ectoparasite infestation and was not related directly to effects of Douglas fir.

References

Council of Europe (1974). Natural Flavouring Substances, Their Sources, and Added Artificial Flavouring Substances. Partial Agreement in the Social and Public Health Field. Strasbourg.

Flavoring Extract Manufacturers' Association (1965). Survey of flavoring ingredients usage levels. *Fd Technol., Champaign* **19** (2), part 2, 155.

Food Chemicals Codex (1972). 2nd Ed. Prepared by the Committee on Specifications, Food Chemicals Codex, of the Committee on Food Protection. National Academy of Sciences–National Research Council Publ. 1406, Washington, DC.

Guenther, E. (1952). *The Essential Oils*. Vol. VI. p. 212. D. Van Nostrand, Inc., Princeton, NJ.

Heston, W. E. (1975). Testing for possible effects of cedar wood shavings and diet on occurrence of mammary gland tumors and hepatomas in C3H-A^{vy} and C3H-A^{vy}fB mice. *J. natn. Cancer Inst.* **54**, 1011.

Kligman, A. M. (1966). The identification of contact allergens by human assay. III. The maximization test. A procedure for screening and rating contact sensitizers. *J. invest. Derm.* **47**, 393.

Kligman, A. M. (1975). Report to RIFM, 14 February.

Kligman, A. M. & Epstein, W. (1975). Updating the maximization test for identifying contact allergens. *Contact Dermatitis* **1**, 231.

McGee, G. (1974). Report to RIFM, 27 September.

Sabine, J. R. (1975). Exposure to an environment containing the aromatic red cedar, *Juniperus virginiana*: procarcinogenic, enzyme-inducing and insecticidal effects. *Toxicology* **5**, 221.

Sabine, J. R., Horton, B. J. & Wicks, M. B. (1973). Spontaneous tumors in C3H-A^{vy} and C3H-A^{vy}fB mice. High incidence in the United States and low incidence in Australia. *J. natn. Cancer Inst.* **50**, 1237.

Urbach, F. & Forbes, P. D. (1974). Report to RIFM, 18 September.

Additional references

Composition

Erdtman, H., Kimland, B., Norin, T. & Daniels, P. J. L. (1968). Chemistry of the Order Pinales. XLIV. The constituents of the pocket resin from Douglas fir *Pseudotsuga menziesii*. *Acta chem. scand.* **22**, 938.

Hergert, H. L. (1960). Chemical composition of tannins and polyphenols from conifer wood and bark. *Forest Prod. J.* **10**, 610.

Snajberk, K., Lee, C. J. & Zavarin, E. (1974). Chemical composition of volatiles from cortical oleoresin of *Pseudotsuga menziesii*. *Phytochemistry* **13**, 185.

Insects

Rudinsky, J. A. (1966). Host selection and invasion by the Douglas-fir beetle, Dendroctonus pseudotsugae, in coastal Douglas-fir forests. *Can. Entomol.* **98**, 98.

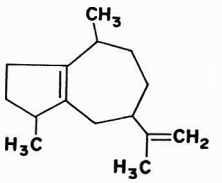
Wellington, W. G. (1969). Effects of three hormonal mimics on mortality, metamorphosis, and reproduction of the western tent caterpillar, *Malacosoma californicum pluviale*. *Can. Entomol.* **101**, 1163.

Micro-organism

Oeriu, S., Moga, V., Bertescu, L., Benesch, H., Cracea, M. & Teodorescu, O. (1959). Cercetari in vederea obtinerii de produse cu actiune tuberculostatica. II. Corelatii intre proprietatile chimice, fizicochimice, structura si proprietatile biologice ale unor produse obtinute din rasini de conifere. *Acad. Rep. Populate Romine, Studii Cercetari Chim.* **7**, 531.

GUAIENE

Synonym: Mainly 1,4-dimethyl-7-isopropenyl-Δ9,10-octahydroazulene.
Structure:



Description and physical properties: A colourless oily liquid.
Occurrence: Found in patchouli oil and other essential oils in small quantities (*Fenaroli's Handbook of Flavor Ingredients*, 1975).
Preparation: By dehydrating guaiol with $KHSO_4$ (*Fenaroli's Handbook of Flavor Ingredients*, 1975).
Uses: In public use since the 1950s.
Concentration in final product (%):

	Soap	*Detergent*	*Creams, lotions*	*Perfume*
Usual	0·02	0·002	0·005	0·1
Maximum	0·15	0·015	0·05	0·4

Analytical data: Gas chromatogram, RIFM no. 76–129; infra-red curve, RIFM no. 76–129.

Status

Guaiene is approved by the FDA for food use (21 CFR 172.515). CAS Registry No. 88–84–6.

Biological data

Acute toxicity. Both the acute oral LD_{50} in rats and the acute dermal LD_{50} in rabbits exceeded 5 g/kg (Moreno, 1976). Guaiene fed in the diet (at 0·40 and 0·50%) over a 10-day period stimulated liver regeneration in partially hepatectomized rats but was ineffective when injected sc at an overall dosage of 2·135 g/kg over a 10-day period (Gershbein, 1977).

Irritation. Guaiene applied full strength to intact or abraded rabbit skin for 24 hr under occlusion was moderately to severely irritating (Moreno, 1976). Tested at 2% in petrolatum, it produced no irritation after a 48-hr closed-patch test on human subjects (Epstein, 1976).

Sensitization. A maximization test (Kligman, 1966; Kilgman & Epstein, 1975) was carried out on 29 volunteers. The material (RIFM no. 76–129) was tested at a concentration of 2% in petrolatum and produced no sensitization reactions (Epstein, 1976).

References

Epstein, W. L. (1976). Report to RIFM, 27 August.
Fenaroli's Handbook of Flavor Ingredients (1975). Edited by T. E. Furia and N. Bellanca, 2nd Ed. Vol. II, p. 226. CRC Press, Cleveland, OH.
Gershbein, L. L. (1977). Regeneration of rat liver in the presence of essential oils and their constituents. *Fd Cosmet. Toxicol.* **15**, 173.
Kligman, A. M. (1966). The identification of contact allergens by human assay. III. The maximization test. A procedure for screening and rating contact sensitizers. *J. invest. Derm.* **47**, 393.
Kligman, A. M. & Epstein, W. (1975). Updating the maximization test for identifying contact allergens. *Contact Dermatitis* **1**, 231.
Moreno, O. M. (1976). Report to RIFM, 31 July.

cis-3-HEXENYL SALICYLATE

Synonym: β,γ-*cis*-Hexenyl salicylate.
Structure: $HO \cdot C_6H_4 \cdot OCO \cdot [CH_2]_2 \cdot CH{:}CH \cdot CH_2 \cdot CH_3$.
Description and physical properties: A colourless oily liquid.
Occurrence: Has apparently not been reported to occur in nature.
Preparation: From *cis*-3-hexenol and salicylic acid by azeotropic-type esterification (Arctander, 1969).
Uses: In public use since the 1960s.

Concentration in final product (%):

	Soap	*Detergent*	*Creams, lotions*	*Perfume*
Usual	0·005	0·0005	0·003	0·08
Maximum	0·05	0·005	0·015	0·5

Analytical data: Gas chromatogram, RIFM no. 74–210; infra-red curve, RIFM no. 74–210.

Status

cis-3-Hexenyl salicylate is not included in the listings of the FDA, FEMA (1965) or the Council of Europe (1974), or in the *Food Chemicals Codex* (1972).

Biological data

Acute toxicity. The acute oral LD_{50} in rats was reported as approximately 5 g/kg and the acute dermal LD_{50} in rabbits exceeded 5 g/kg (Moreno, 1975).

Irritation. cis-3-Hexenyl salicylate applied full strength to intact or abraded rabbit skin for 24 hr under occlusion was moderately irritating (Moreno, 1975). Tested at 3% in petrolatum, it produced no irritation after a 48-hr closed-patch test on human subjects (Kligman, 1975).

Sensitization. A maximization test (Kligman, 1966; Kligman & Epstein, 1975) was carried out on 25 volunteers. The material (RIFM no. 74–210) was tested at a concentration of 3% in petrolatum and produced no sensitization reactions (Kligman, 1975).

References

Arctander, S. (1969). *Perfume and Flavor Chemicals (Aroma Chemicals)*. Vol. I, no. 1628. S. Arctander, Montclair, NJ.

Council of Europe (1974). Natural Flavouring Substances, Their Sources, and Added Artificial Flavouring Substances. Partial Agreement in the Social and Public Health Field. Strasbourg.

Flavoring Extract Manufacturers' Association (1965). Survey of flavoring ingredient usage levels. *Fd Technol., Champaign* **19** (2), part 2, 155.

Food Chemicals Codex (1972). 2nd Ed. Prepared by the Committee on Specifications, Food Chemicals Codex, of the Committee on Food Protection. National Academy of Sciences–National Research Council Publ. 1406, Washington, DC.

Kligman, A. M. (1966). The identification of contact allergens by human assay. III. The maximization test. A procedure for screening and rating contact sensitizers. *J. invest. Derm.* **47**, 393.

Kligman, A. M. (1975). Report to RIFM, 15 January.

Kligman, A. M. & Epstein, W. (1975). Updating the maximization test for identifying contact allergens. *Contact Dermatitis* **1**, 231.

Moreno, O. M. (1975). Report to RIFM, 3 February.

METHYL HEPTINE CARBONATE

Synonym: Methyl 2-octynoate.
Structure: $CH_3 \cdot [CH_2]_4 \cdot C \vdots C \cdot OCO \cdot CH_3$.
Description and physical properties: EOA Spec. no. 145.
Occurrence: Has apparently not been reported to occur in nature.
Preparation: May be synthesized from heptaldehyde via heptyne and heptyne carboxylic acid. The acid is then esterified.
Uses: In public use since the 1900s. Use in fragrances in the USA amounts to approximately 3000 lb/yr.

Concentration in final product (%):

	Soap	*Detergent*	*Creams, lotions*	*Perfume*
Usual	0·0025	0·0003	0·001	0·04
Maximum	0·015	0·002	0·005	0·2

Status

Methyl heptine carbonate was given GRAS status by FEMA (1965), is approved by the FDA for food use (21 CFR 172.515) and was included by the Council of Europe (1974), at a level of 4 ppm, in the list of artificial flavouring substances that may be added to foodstuffs without hazard to public health. The *Food Chemicals Codex* has a monograph on methyl heptine carbonate. CAS Registry No. 111-12-6.

Biological data

Acute toxicity. The acute oral LD_{50} in rats has been reported as 1·53 g/kg (Bär & Griepentrog, 1967) and as 2·5 g/kg (2·0–3·0 g/kg) (Moreno, 1972), and the acute dermal LD_{50} in rabbits was reported as 3·3 g/kg (Moreno, 1972).

Chronic toxicity. In rats fed 0·1 or 0·5% methyl heptine carbonate in the diet for 2 yr, the higher dose caused growth inhibition but no other adverse effects were found (Bär & Griepentrog, 1967).

Invertebrates. When applied topically at a dose of 0·2 µl to the larvae of the house fly (*Musca domestica*), methyl heptine carbonate produced 100% mortality (Quraishi, 1972).

Irritation. Methyl heptine carbonate applied full strength to intact or abraded rabbit skin for 24 hr under occlusion was moderately irritating (Moreno, 1972). When tested at 2% in petrolatum, it produced no irritation after a 48-hr closed-patch test on 12 different panels of human subjects (Epstein, 1974, 1975 & 1976; Kligman, 1974, 1975 & 1977).

Sensitization. Maximization tests (Kligman, 1966; Kligman & Epstein, 1975) were carried out on groups of volunteers in several different panels, each of approximately 25 subjects. Of the samples of methyl heptine carbonate tested (all at a concentration of 2% in petrolatum), the numbers of sensitization reactions produced in these groups were: seven with sample no. 71–50 (Kligman, 1972), none with no. 74–157R(7G) (Epstein, 1974), three with no. 74–270 (Kligman, 1974), ten with no. 74–279 (Kligman, 1975), seven with no. 74–278 (Kligman, 1975), four with no. 75–143 (Epstein, 1975), five with no. 74–278R(7) (Epstein, 1975), one (only questionably positive) with no. 74–157R(O) (Epstein, 1975), four with no. 75–151 (Epstein, 1975), none (in 21 subjects) with no. 75–162, a stabilized methyl heptine carbonate (Epstein, 1975), seven with no. 75–197 (Epstein, 1976) and five with no. 75–198 (Epstein, 1976). The stabilized sample no. 75–162, which produced no sensitization reactions, was then stored under refrigeration for approximately 12 months, after which it was retested, renumbered as 75–162R(O), again at 2% in petrolatum, and produced eight sensitization reactions (Kligman, 1977).

The following summary of these results identifies the samples in more detail:

Sample no.	*Positive reactions**	*Sample identity*
71–50	7/25	Company 'A'.
74–157R(7G)	0/25	Company 'B'.
74–270	3/25	Company 'B', new sample; same batch as 74–157R(7G). Tested 3 months after the sample of 74–157R(7G) had been tested.
74–279	10/25	New sample; same batch as 74–157R(7G). Tested several months after the sample of 74–157R(7G) had been tested.
74–278	7/25	Same as sample 71–50. Company 'A'.

*No. of positive sensitization reactions/no. tested.

75–143	4/25	Company 'A', new process.
74–278R(7)	5/22	Same as sample 71–50. Company 'A'.
74–157R(O)	1/24	Repeat of sample 74–157R(7G) tested 8 months later; gave one questionable reaction. Company 'B'.
75–151	4/24	Company 'B', new sample.
75–162	0/21	MHC stabilized with BHA.
75–197	7/25	MHC stabilized; Company 'B'. Held for 3 months unopened before testing.
75–198	5/26	Company 'B'. Held for 3 months unopened before testing.
75–162R(O)	8/25	Repeat of sample 75–162, after storage under refrigeration for approximately 12 months.

IFRA data: The following information was provided in an unpublished communication from IFRA dated 1978:

> Animal (guinea-pig) testing indicated a potential for sensitization in concentrations at or above 1% on skin. Human repeated-insult patch tests (HRIPT) produced two sensitization reactions in a panel of 41 when the material was tested at a concentration of 0·25% in ethanol, but no reactions occurred at 0·1 or 0·05% in panels of 42 and 40 respectively. Testing (HRIPT) at concentrations, on the skin, of 0·0002 and 0·001% produced no sensitization reactions in panels of 53 and ten (of which four were sensitive), respectively.

Comment: Methyl heptine carbonate when freshly distilled from a freshly prepared sample, whatever the means of preparation, does not appear to sensitize. On standing, however, it appears to accumulate a very powerful sensitizing component. This component has so far defied all analytical detection methods and consequently must be present in miniscule amounts and must be a very powerful sensitizer. It was first thought that since this develops with ageing of the sample, peroxidation might be the explanation. However, in samples exposed to light and having oxygen bubbled through for some time, no detectable accumulation of peroxide was observed, nor did the addition of antioxidants stabilize the material for more than 2 months. Efforts at quenching have been unsuccessful. One sample that sensitized (sample no. 75–162) did not produce reactions after being freshly distilled, but regained its sensitizing potential on standing.

A laboratory sample of a homologue, ethyl heptine carbonate, was prepared to determine its suitability as a possible replacement. When tested at 2% in petrolatum, this too produced sensitization reactions.

References

Bär, F. u. Griepentrog, F. (1967). Die Situation in der gesundheitlichen Beurteilung der Aromatisierungsmittel für Lebensmittel. *Medizin Ernähr.* **8**, 144.

Council of Europe (1974). Natural Flavouring Substances, Their Sources, and Added Artificial Flavouring Substances. Partial Agreement in the Social and Public Health Field. List 1, no. 481, p. 225. Strasbourg.

Epstein, W. L. (1974). Report to RIFM, 23 July.

Epstein, W. L. (1975). Reports to RIFM, 5 and 28 March, 16 April, 11 July and 22 December.

Epstein, W. L. (1976). Report to RIFM, 23 July.

Flavoring Extract Manufacturers' Association (1965). Survey of flavoring ingredient usage levels. No. 2729. *Fd Technol., Champaign* **19** (2), part 2, 155.

Food Chemicals Codex (1972). 2nd Ed. Prepared by the Committee on Specifications, Food Chemicals Codex, of the Committee on Food Protection. p. 529. National Academy of Sciences–National Research Council Publ. 1406, Washington, DC.

Kligman, A. M. (1966). The identification of contact allergens by human assay. III. The maximization test. A procedure for screening and rating contact sensitizers. *J. invest. Derm.* **47**, 393.

Kligman, A. M. (1972). Report to RIFM, 14 March.

Kligman, A. M. (1974). Reports to RIFM, 8 January and 18 October.

Kligman, A. M. (1975). Report to RIFM, 8 January.

Kligman, A. M. (1977). Report to RIFM, 14 February.

Kligman, A. M. & Epstein, W. (1975). Updating the maximization test for identifying contact allergens. *Contact Dermatitis* **1**, 231.

Moreno, O. M. (1972). Reports to RIFM, 1 and 5 May.

Quraishi, M. S. (1972). Toxic and teratogenic effects of esters of saturated and unsaturated fatty acids on house fly larvae (Diptera: Muscidae). *Can. Entomol.* **104**, 1505.

PHENYLACETALDEHYDE

Synonym: α-Tolualdehyde.
Structure: $C_6H_5 \cdot CH_2 \cdot CHO$.
Description and physical properties: EOA Spec. no. 192.
Occurrence: Has been identified among the constituents of several essential oils, including those of neroli, narcissus, magnolia, lily, rose, tea, *Citrus sinensis* leaves and flowers and leaves of other citrus species (*Fenaroli's Handbook of Flavor Ingredients*, 1975).
Preparation: By Darzen glycidic ester synthesis from benzaldehyde.
Uses: In public use before 1920. Use in fragrances in the USA amounts to approximately 18,000 lb/yr.
Concentration in final product (%):

	Soap	*Detergent*	*Creams, lotions*	*Perfume*
Usual	0·005	0·0005	0·003	0·1
Maximum	0·1	0·01	0·03	0·2

Analytical data: Gas chromatogram, RIFM no. 70–76; infra-red curve, RIFM no. 70–76.

Status

Phenylacetaldehyde (PAA) was given GRAS status by FEMA (1965), is approved by the FDA for food use (21 CFR 172.515) and was listed by the Council of Europe (1974) with an ADI of 1 mg/kg. The *Food Chemicals Codex* (1972) has a monograph on PAA. CAS Registry No. 122–78–1.

Biological data

Acute toxicity. The acute oral LD_{50} in rats, mice and guinea-pigs was reported as 3·89 g/kg (32·4 mmol/kg) following administration as a 20–40% solution in sunflower-seed oil, no difference being demonstrated between the three species (Zaitsev & Rakhmanina, 1974). The acute oral LD_{50} in rats has also been reported as 1·55 g/kg (1·0–2·4 g/kg) and the acute dermal LD_{50} in rabbits has been reported as > 5 g/kg (Moreno, 1977).

Irritation. PAA tested at 2% in petrolatum was irritating to two of 25 human subjects after a 48-hr closed-patch test (Kligman, 1971). Tested again at 2% in petrolatum, it produced no irritation after 48-hr closed-patch tests on two different panels of human subjects (Epstein, 1973; Kligman, 1971).

Sensitization. When tested on guinea-pig skin by the method of Buehler (1965), 5·0% PAA produced sensitization reactions to a challenge dose of 1·0% in vaseline (Majeti & Suskind, 1976/1977; Suskind & Majeti, 1976). Tested by the same method, 2% PAA also produced several sensitization reactions (Briggs, 1974). A guinea-pig patch test using the Maguire method was carried out on five guinea-pigs with 4% PAA and produced sensitization reactions in all five (H. N. Prince, personal communication to RIFM, 1972).

Patch tests on 275 patients indicated that PAA was a skin irritant at 10% in alcohol and a sensitizer, at 10 or 0·5% in alcohol, to further applications of 0·01% PAA solutions (Fregert, 1970). The author questioned whether PAA should be included in perfumes and flavouring agents owing to its ability to sensitize.

Four maximization tests (Kligman 1966; Kligman & Epstein, 1975) were carried out on four different panels of human subjects using the same material (RIFM no. 1463070) at 2% in petrolatum. The first test produced 11 sensitization reactions in 25 subjects (Kligman, 1971), the second four in 25 (Kligman, 1971), the third 12 in 23 (Epstein, 1973), and the fourth two in 25 (Maibach, 1971). These last two reactions were confirmed by a 'use test'. Of the 12 reactors identified in the third of these tests (Epstein, 1973), seven were still available for testing 4 months later and were challenged with a 50/50 mixture of PAA and phenyl ethyl alcohol (PEA) at 2% in petrolatum and with a fresh sample of PAA at 2% in petrolatum. Both of the materials evoked sensitization responses in the test subjects (Epstein, 1973).

PAA was also tested by the repeated-insult patch procedure: no reactions were produced either at 2% using eleven 24-hr exposures (Draize, 1959) on 56 human subjects (Maibach, 1971) or at 2% using fifteen 24-hr exposures (modified Draize, 1959) on 50 human subjects (Shelanski, 1971).

Quenching studies: In the course of maximization testing in human subjects, three instances arose in which an individual aldehyde (PAA, citral or cinnamic aldehyde) occurring widely in nature proved to be a skin sensitizer. However, the essential oil in which the aldehyde occurred naturally did not induce sensitization reactions, although the aldehyde was present in concentrations as high as 85%. It appeared that some other component(s) of the natural oil inhibited the induction or expression of sensitization. As a test of this hypothesis, several terpenes and alcohols found along with the particular aldehyde in the natural composition were combined with each of the aldehydes in question. It appears now to be a consistent finding that each of these aldehydes, although produc-

ing sensitization reactions when applied alone, produces no sensitization reactions in selected simple mixtures with other compounds (Opdyke, 1976). The results of quenching tests using the maximization procedure (Kligman, 1966; Kligman & Epstein, 1975) are summarized in Table 1.

Table 1. *Results of quenching tests on mixtures of phenylacetaldehyde with other essential-oil components*

Second test material	Relative proportions*	Overall concentration (%)	Results of sensitization test†
Ethanol	1:1	4	+
Benzyl alcohol	1:1	4	+
	1:1‡	4	+
Mixed citrus terpenes	4:1	3	+
d-Limonene	4:1	3	+
Phenylethyl alcohol	1:1	4	–
	1:1‡	4	–
Dipropylene glycol	1:1	4	–
Eugenol	1:1	4	+

*Ratio (w/w) of phenylacetaldehyde to second test material. Each mixture was tested by the maximization procedure (Kligman, 1966; Kligman & Epstein, 1975).

†Where + indicates some reactions in the test group (generally 25 subjects) and – indicates no reactions in the group.

‡Repeat test.

Animals induced with a combination of PAA and PEA showed, upon challenge, skin reactions to PAA as well as to the combination of PAA and PEA. The highest non-irritating concentrations of PAA for guinea-pig skin were 1% in vaseline and 2% in acetone (Majeti & Suskind, 1976/1977; Suskind & Majeti, 1976).

Penetration. Preliminary experiments with isolated human epidermis in transfer chambers indicated that PAA penetrated epidermis rapidly; its flux was not altered by at least two surfactants but was increased significantly by PEA (H. Saylor, personal communication 1976).

Pharmacology. PAA induced CNS depression in mice and chicks when administered ip at high doses (Sabelli & Giardina, 1970). When PAA was administered im to frogs at 0·5–4 mg/kg, it failed to have an effect on the transmembranal resting potential of liver cells (Orozco & Sabelli, 1970).

Cytotoxicity. PAA inhibited the growth of ascites sarcoma BP 8 cells by 100% at a concentration of 1 mM and by 76% at 0·1 mM but was ineffective at 0·01 mM (Pilotti, Ancker, Arrhenius & Enzell, 1975).

Isolated tissue. PAA was shown to stimulate [1-^{14}C]glucose oxidation by bovine anterior-pituitary slices (Barondes, 1962).

Vertebrate enzymes and proteins. PAA was an inhibitor of Na^+-, K^+- and Mg^{++}-ATPase and the K^+-dependent *p*-nitrophenylphosphatase of mouse brain in the absence of cysteine or mercaptoethanol (Erwin, Kim & Anderson, 1975). It was found to be a substrate for phenobarbital-induced cytoplasmic aldehyde dehydrogenase from rat liver (Koivula & Koivusalo, 1975), and Tabakoff, Anderson & Alivisatos (1973) reported that it was a substrate for rat-brain aldehyde dehydrogenase and was the oxidation product derived from phenylethylamine in the presence of rat-liver monoamine oxidase. When tested as a substrate for glyceraldehyde-3-phosphate dehydrogenase from rabbit muscle, PAA was oxidized to the acid (Fife & Rikihisa, 1970).

PAA stimulated transhydrogenase activity in pig anterior-pituitary homogenate (McGuire & Pesch, 1962). The products of the non-enzymic reaction of PAA with NAD^+ from pig brain were postulated to be adducts between the aldehyde and the nicotinamide moiety of the coenzyme (Duncan & Tipton, 1971a). PAA was a substrate for aldehyde dehydrogenase from bovine brain mitochondria (Erwin & Deitrich, 1966) and from pig brain (Duncan & Tipton, 1971b). At concentrations above 5×10^{-4} M, it was found to inhibit dissociation of the horse-liver alcohol dehydrogenase-DPN^+ complex (Wratten & Cleland, 1965).

Aromatic aldehydes, including PAA, were oxidized by peroxidase under aerobic conditions (Duran, Zinner, Vidigal & Cilento, 1977). PAA activated the blood-clotting factor, Factor I (Nour-Eldin, 1967), produced a rapid thrombin-fibrinogen reaction and accelerated stypven clotting time (Nour-Eldin, 1968).

Invertebrates. PAA was definitely attractive to both sexes of the adult cabbage looper, *Trichoplusia ni* (Creighton, McFadden & Cuthbert, 1973).

Micro-organisms

Bacteria. Using *Achromobacter eurydice* isolated from soil, the pathway by which phenyl pyruvate is metabolized to phenyl acetate through PAA was clarified; PAA dehydrogenase required NAD^+ as a cofactor (Asakawa, Wada & Yamano, 1968).

Viruses. PAA was found to have no inhibitory activity against vesicular stomatitis virus, as indicated by the development of plaques on porcine-kidney, monkey-kidney and canine-kidney cell cultures (Kremzner & Harter, 1970). A variety of bacteriophages was inactivated by PAA and PEA under conditions normally used to inhibit the synthesis of DNA with PEA, and there were indications that these inhibitors had an effect on protein (Mendelson & Fraser, 1965).

Fungi. Growth of *Rhizoctonia solani* and formation of a toxin containing phenylacetic acid readily occurred in the presence of PAA (Kohmoto, Fukui, Mizuno & Nishimura, 1973).

References

Asakawa, T., Wada, H. & Yamano, T. (1968). Enzymic conversion of phenylpyruvate to phenylacetate. *Biochim. biophys. Acta* **170,** 375.

Barondes, S. H. (1962). The influence of neuroamines on the oxidation of glucose by the anterior pituitary. *J. biol. Chem.* **237,** 204.

Briggs, G. B. (1974). Report to RIFM, 9 August.

Buehler, E. V. (1965). Delayed contact hypersensitivity in the guinea-pig. *Archs Derm.* **91,** 171.

Council of Europe (1974). Natural Flavouring Substances, Their Sources, and Added Artificial Flavouring Substances. Partial Agreement in the Social and Public Health Field. List 1, no. 116, p. 148. Strasbourg.

Creighton, C. S., McFadden, T. L. & Cuthbert, E. R. (1973). Supplementary data on phenylacetaldehyde: an attractant for Lepidoptera. *J. econ. Ent.* **66,** 114.

Draize, J. H. (1959). Dermal toxicity. In *Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics.* p. 52. Association of Food and Drug Officials of the United States, Austin, TX.

Duncan, R. J. S. & Tipton, K. F. (1971a). Purification and properties of the NAD-linked aldehyde dehydrogenase from pig brain. *Eur. J. Biochem.* **22,** 257.

Duncan, R. J. S. & Tipton, K. F. (1971b). The kinetics of pig brain aldehyde dehydrogenase. *Eur. J. Biochem.* **22,** 538.

Duran, N., Zinner, K., Vidigal, C. C. C. & Cilento, G. (1977). Generation of electronically excited aromatic aldehydes in the peroxidase catalysed aerobic oxidation of aromatic acetaldehydes. *Biochem. biophys. Res. Commun.* **74,** 1146.

Epstein, W. L. (1973). Report to RIFM, 29 June and 9 October.

Erwin, V. G. & Deitrich, R. A. (1966). Brain aldehyde dehydrogenase localization, purification and properties. *J. biol. Chem.* **241,** 3533.

Erwin, V. G., Kim, J. & Anderson, A. D. (1975). Effects of aldehydes on sodium-plus-potassium ion-stimulated adenosine triphosphatase of mouse brain. *Biochem. Pharmac.* **24,** 2089.

Fenaroli's Handbook of Flavor Ingredients (1975). Edited by T. E. Furia and N. Bellanca. 2nd Ed. Vol. II, p. 471. CRC Press Co., Cleveland, OH.

Fife, T. H. & Rikihisa, T. (1970). Reaction of glyceraldehyde 3-phosphate dehydrogenase with aliphatic aldehydes. *Biochemistry, N.Y.* **9,** 4064.

Flavoring Extract Manufacturers' Association (1965). Survey of flavoring ingredient usage levels. No. 2874. *Fd Technol., Champaign* **19** (2), part 2, 155.

Food Chemicals Codex (1972). 2nd Ed. Prepared by the Committee on Specifications, Food Chemicals Codex, of the Committee on Food Protection. p. 604. National Academy of Sciences–National Research Council Publ. 1406, Washington, DC.

Fregert, S. (1970). Sensitization to phenylacetaldehyde. *Dermatologica* **141,** 11.

Kligman, A. M. (1966). The identification of contact allergens by human assay. III. The maximization test. A procedure for screening and rating contact sensitizers. *J. invest. Derm.* **47,** 393.

Kligman, A. M. (1971). Reports to RIFM, 20 April and 9 June.

Kligman, A. M. & Epstein, W. (1975). Updating the maximization test for identifying contact allergens. *Contact Dermatitis* **1,** 231.

Kohmoto, K., Fukui, R., Mizuno, M. & Nishimura, S. (1973). Pathochemical studies on Rhizoctonia disease. III. Characteristic production of Rhizoctonia toxins and their *in vivo* toxicity. *J. Fac. Agric., Tottori Univ.* **8,** 21.

Koivula, T. & Koivusalo, M. (1975). Partial purification and properties of a phenobarbital-induced aldehyde dehydrogenase of rat liver. *Biochim. biophys. Acta* **410,** 1.

Kremzner, L. T. & Harter, D. H. (1970). Antiviral activity of oxidized polyamines and aldehydes. *Biochem. Pharmac.* **19,** 2541.

Maibach, H. I. (1971). Report to RIFM, September.

Majeti, V. A. & Suskind, R. R. (1976/1977). The sensitizing activity of three aroma aldehydes and inhibition by specific chemical agents. Reports to RIFM, 16 January 1976, 7 January 1977 and 1 November 1977.

McGuire, J. & Pesch, L. (1962). Control of glucose oxidation in anterior pituitary by hormonally sensitive pyridine nuceleotide transhydrogenase. *Proc. natn. Acad. Sci. U.S.A.* **48,** 2157.

Mendelson, N. H. & Fraser, D. (1965). Physical effects of the deoxyribonucleic acid inhibitor beta-phenethyl alcohol. *Biochim. biophys. Acta* **102,** 559.

Moreno, O. M. (1977). Report to RIFM, 7 February.

Nour-Eldin, F. (1967). Chemical activation of blood-clotting factors and thrombosis. *Nature, Lond.* **214,** 1362.

Nour-Eldin, F. (1968). Phenols and blood coagulation. *J. biomed. Mater. Res.* **2,** 23.

Opdyke, D. L. J. (1976). Inhibition of sensitization reactions induced by certain aldehydes. *Fd Cosmet. Toxicol.* **14,** 197.

Orozco, A. & Sabelli, H. (1970). Effect of catechol amines and nicotine on the transmembranal potential of frog liver cells. *Experientia* **26,** 48.

Pilotti, A., Ancker, K., Arrhenius, E. & Enzell, C. (1975). Effects of tobacco and tobacco smoke constituents on cell multiplication *in vitro*. *Toxicology* **5**, 59.
Sabelli, H. C. & Giardina, W. J. (1970). CNS effects of the aldehyde products of brain monoamines. *Biol. Psychiat.* **2**, 119.
Shelanski, M. V. (1971). Report to RIFM, 30 August.
Suskind, R. R. & Majeti, V. A. (1976). Occupational and environmental allergic problems of the skin. *J. Derm.* **3**, 3.
Tabakoff, B., Anderson, R. & Alivasatos, G. A. S. (1973). Enzymic reduction of biogenetic aldehydes in brain. *Molec. Pharmacol.* **9**, 428.
Wratten, C. C. & Cleland, W. W. (1965). Kinetic studies with liver alcohol dehydrogenase. *Biochemistry, N.Y.* **4**, 2442.
Zaitsev, A. N. & Rakhmanina, N. L. (1974). Toxic properties of phenylethanol and cinnamic alcohol derivatives. *Vop. Pitan.* no. 5, p. 48.

PIMENTA BERRY OIL

Synonym: Oil allspice.

Description and physical properties: EOA Spec. no. 255. The volatile oil of pimenta berry contains about 70% eugenol as well as eugenol methyl ether, cineol, *l*-α-phellandrene and caryophyllene (*Merck Index*, 1976). It has also been reported that pimenta berry oil has a 65–90% phenol content, as eugenol, as well as containing phellandrene and caryophyllene (Appell, 1968).

Occurrence: Found in the fruit (berries) of *Pimenta officinalis* Lindley (Fam. Myrtaceae).

Preparation: By steam distillation of the dried fruit of *P. officinalis* Lindley.

Uses: In public use before the 1890s. Use in fragrances in the USA amounts to approximately 1000 lb/yr.

Concentration in final product (%):

	Soap	*Detergent*	*Creams, lotions*	*Perfume*
Usual	0·02	0·002	0·01	0·3
Maximum	0·2	0·02	0·07	1·2

Status

Pimenta berry oil was given GRAS status by FEMA (1965), is approved by the FDA for food use (GRAS) and was included by the Council of Europe (1974) in the list of substances, spices and seasonings deemed admissible for use, with a possible limitation of the active principle in the final product. The *Food Chemicals Codex* (1972) has a monograph on pimenta berry oil.

Biological data

Irritation. Undiluted pimenta berry oil applied to the backs of hairless mice and swine was not irritating (Urbach & Forbes, 1976). Tested at 8% in petrolatum it produced no irritation after a 48-hr closed-patch test on human subjects (Epstein, 1976).

Sensitization. A maximization test (Kligman, 1966; Kligman & Epstein, 1975) was carried out on 32 volunteers. The material (RIFM no. 75–183) was tested at a concentration of 8% in petrolatum and produced no sensitization reactions (Epstein, 1976).

Phototoxicity. No phototoxic reactions were reported for undiluted pimenta berry oil on hairless mice and swine (Urbach & Forbes, 1976).

Percutaneous absorption. Oil of pimenta was not absorbed within 2 hr by the intact shaved, abdominal skin of the mouse (Meyer & Meyer, 1959).

References

Appell, L. (1968). Physical foundations in perfumery. VI. Volatility of the essential oils. *Am. Perfumer Cosm.* **83**, 37.

Council of Europe (1974). Natural Flavouring Substances, Their Sources, and Added Artificial Flavouring Substances. Partial Agreement in the Social and Public Health Field. List N(1) Ser. I(b). no. 335, p. 89. Strasbourg.

Epstein, W. L. (1976). Report to RIFM, 30 March.

Flavoring Extract Manufacturers' Association (1965). Survey of flavoring ingredient usage levels. No. 2018. *Fd Technol., Champaign* **19** (2), part 2, 155.

Food Chemicals Codex (1972). 2nd Ed. Prepared by the Committee on Specifications, Food Chemicals Codex, of the Committee on Food Protection. p. 618. National Academy of Sciences–National Research Council Publ. 1406. Washington, DC.

Kligman, A. M. (1966). The identification of contact allergens by human assay. III. The maximization test. A procedure for screening and rating contact sensitizers. *J. invest. Derm.* **47**, 393.

Kligman, A. M. & Epstein, W. (1975). Updating the maximization test for identifying contact allergens. *Contact Dermatitis* **1**, 231.

Merck Index (1976). *An Encyclopedia of Chemicals and Drugs.* 9th Ed., no. 6647. Merck & Co., Inc., Rahway, NJ.

Meyer, F. u. Meyer, E. (1959). Percutane Resorption von ätherischen Ölen und ihren Inhaltsstoffen. *Arzneimittel-Forsch.* **9**, 516.

Urbach, F. & Forbes, P. D. (1976). Report to RIFM, 9 February.

Additional reference

Invertebrates

Oishi, K., Mori, K. & Nishiura, Y. (1974). Food hygienic studies on *Anisakinae* larvae. V. Effects of some spice essential oils and food preservatives on the mortality of *Anisakinae* larvae. *Nippon Suisan Gakkaishi* **40**, 1241.

STEARIC ACID

Synonyms: Octadecanoic acid; heptadecane-1-carboxylic acid.
Structure: $CH_3 \cdot [CH_2]_{16} \cdot COOH$.
Description and physical properties: Merck Index (1976).
Occurrence: The principal natural source of stearic acid is animal fat (triglyceride) from which the free acid, mixed with other fatty acids and glycerol, is obtained by hydrolysis. Stearic acid also occurs in esterified form in other classes of lipids, including cholesterol esters and phospholipids. To a lesser extent, it is present in a mixture of free fatty acids in blood and a variety of tissues.
Preparation: By hydrogenation of oleic acid or cottonseed oil (Arctander, 1969).
Uses: Concentration in final product (%):

	Soap	*Detergent*	*Creams, lotions*	*Perfume*
Usual	0·01	0·001	0·002	0·2
Maximum	0·1	0·01	0·03	0·7

Status

Stearic acid was given GRAS status by FEMA (1965), is approved by the FDA for food use (21 CFR 121.1070) and was included by the Council of Europe (1974), at a level of 4000 ppm, in the list of artificial flavouring substances that may be added to foodstuffs without hazard to public health. Both the *Food Chemicals Codex* (1972) and the *United States Pharmacopeia* (1975) have a monograph on stearic acid. CAS Registry No. 57–11–4.

Biological data

Acute toxicity. Both the acute oral LD_{50} in rats and the acute dermal LD_{50} in rabbits exceeded 5 g/kg (Moreno, 1976). When single doses of stearic acid were administered ip to mice at levels of 1·37, 2·75, 5·5, 11, 22 and 44 μmol, no deaths occurred but there was significant body-weight depression at the 44-μmol dose level, and peritoneal adhesions occurred at 2·7 μmol and higher doses (Hardegree & Kirschstein, 1968). The iv LD_{50}, when stearic acid was injected as a 0·2% emulsion in cottonseed oil, buffer and emulsifiers, was 23 mg/kg in mice and 21·5 mg/kg in rats (Orö & Wretlind, 1961). When doses near the LD_{50} were injected, these animals went into convulsions and collapsed on their sides; respiration ceased within 1–2 min, sometimes after hyperpnoea.

To determine the effects of stearic acid on serum and intestinal alkaline phosphatase, rats were fasted for 4 days and were then fed 0·35 mmol stearic acid and killed after 24 hr; a slight but statistically insignificant effect on alkaline phosphatase was demonstrated (Dickie, Robinson & Tuba, 1955).

In rats fed high-fat diets, the stearic/linoleic ratio correlated closely with the severity of thrombosis produced by injection of Gram-negative endotoxin, stearic acid being thrombogenic while linoleic acid counteracted its effects (Gautheron & Renaud, 1972). Serum cholesterol levels in these rats were related to the degree of saturation of the dietary fat, the clotting time of the blood was decreased, and a significant correlation was found between the decrease in clotting time and the severity of the thrombosis. The iv infusion of stearic acid to rabbits also decreased the clotting time of the blood (Tachikawa, 1974). In rabbits injected into the jugular vein with a 10-ml/kg dose of a 5% albumin solution containing 1·4–2·1 mequiv free fatty acid/litre (including a high proportion of stearic acid), over a 5-min period, toxic signs included an increase in the rate and depth of respiration, and the development of thrombi, in the lungs in eight and in jugular-vein segments in ten of the 15 animals treated (Hoak, Connor & Warner, 1966). Whole-blood silicone clotting time was significantly shortened in animals receiving injections of the albumin–stearic acid mixture (Hoak *et al.* 1966).

In the cat, 0·2 ml stearic acid emulsion/kg given iv had a hypotensive effect on the systemic circulation, but raised the pulmonary arterial pressure and had a negatively inotropic effect on the heart (Orö & Wretlind, 1961). More than 5 mg injected into a cat caused apnoea, a fall in blood pressure, convulsions and death (Orö & Wretlind, 1961).

A 0·1% solution of stearic acid injected as the sodium salt into a foreleg vein of anaesthetized dogs in a dose of 10 ml/kg over a 5-min period killed eight of the nine dogs within 3–5 min (Connor, Hoak & Warner, 1963). ECG monitoring revealed an initial increase in heart rate, followed by a profound depression of the ST segment, and later an irregular cardiac rhythm and periods of asystole. A loud systolic murmur could be heard at about the time the ECG changes appeared. At autopsy, large thrombi were present in the inferior and superior vena cavae and in the right heart extending into the pulmonary artery. With a 0·01% sodium stearate solution, thrombosis and death did not occur. When the infusion time of the 0·1% solution was increased from 5 to 30–40 min, no toxic signs appeared, but a 10-min infusion still caused massive thrombosis and death.

The thrombosis produced in dogs in this way could be prevented by pretreatment with heparin, or partially prevented by administration of warfarin, but the dogs died within 10 min of the 0·1% infusion (at 10 ml/kg), apparently of acute myocardial failure (Hoak, Connor, Eckstein & Warner, 1964). Generalized thrombosis and death did not occur when the stearic acid was incubated with bovine albumin before injection or when it was injected into a branch of the portal vein. Ducks deficient in clotting factors died suddenly following injections of stearic acid into a systemic vein, but only occasional thrombi were noted. In summary, when stearic acid was injected into the systemic circulation, the effects produced were a thrombotic state, resulting from Hageman-factor activation and platelet agglutination, together with acute heart failure, which occurred even in the absence of thrombosis (Hoak *et al.* 1964).

Subacute toxicity. In more prolonged experiments in which stearic acid was applied to the skin and eyes of mice and rats or given orally (dose unstated) no toxic or cumulative action was shown (Komarova, 1976).

Rats fed a high-fat diet, which also contained 5% stearic acid, for 6 wk showed a decrease in clotting time, moderate hyperlipaemia and severe phlebothrombosis, which was initiated by iv injection of a *Salmonella typhosa* lipopolysaccharide in saline; multiple red hepatic infarcts were noted (Renaud, 1968 & 1969). After 9 wk on a high-fat diet containing 6% stearic acid, rats developed a high degree of aortic atherosclerosis and the injection of *S. typhosa* endotoxin into animals on this diet induced a high mortality and severe thrombosis (Renaud, 1968). Hypercoagulation under these conditions has been related to increased incorporation of stearic acid from dietary fat into phosphatide fractions (Gautheron & Renaud, 1972).

In rats fed 3000 ppm stearic acid orally for approximately 30 wk, no significant pathological lesions were seen, but anorexia and increased mortality were reported and severe pulmonary infection was also observed (Deichmann, Radomski, Macdonald, Kascht & Erdmann, 1958). A slight rise in plasma cholesterol was noted in rats given 15% stearic acid orally for 8 wk with or without DL-methionine and choline chloride supplementation, but liver cholesterol and weight gain were normal (Janssen, 1958). Stearic acid fed to male rats at 5% in the diet for 6 wk was found to be the next to least hyperlipaemic of the fatty acids and to produce the shortest plasma clotting time; low triglyceride levels were also found (Popjak, Holloway, Bond & Roberts, 1969). The stearic acid group showed more severe thromboses than the groups given other fatty acids. Stearic acid fed as butter (14·6% stearic acid) for 9 wk or as cocoa butter (35% stearic acid) for 18 wk was again the most thrombogenic, the severity being 2·1 on a scale of 0·3 (Popjak *et al.* 1969).

There was no change in growth rate or feed utilization in chicks fed 5% stearic acid with or without a dispersing agent for 4 wk, but fat in the dry faeces increased from 2·63 to 9·01% (Sunde, 1956).

In rabbits fed an atherogenic diet consisting of 6% corn oil with 2% cholesterol plus 0·25–0·50% stearic acid for 2 months and then killed, determination of cholesterol levels in plasma and liver and examination of atheromata in thoracic aortae and aortic arches showed that the diet enriched with stearic acid had a more pronounced atherogenic effect than a diet containing only corn oil and cholesterol (Kritchevsky & Tepper, 1961).

Osumi, Amano, Okegawa & Shimamoto (1966) fed a low-fat basal diet supplemented with 1% cholesterol and 10% fatty acids to rabbits for 12 wk. One group of animals received 5% ethyl linoleate and 5% stearic acid (S,L group) and another 10% stearic acid (S group). Neither group differed from the controls in body-weight gain or electrocardiograms, but both showed progressive increases in serum cholesterol and serum and thoracic-aorta phospholipids. Liver phospholipid levels were markedly decreased in the S group, in which atheromatous changes of the aorta and kidney were marked, and similar atheromatous changes were noted in the liver and spleen of the S,L group.

Irritation. Stearic acid applied full strength to intact or abraded rabbit skin for 24 hr under occlusion was moderately irritating (Moreno, 1976). Tested as a follicular keratogenic agent by application to the external ear canal of the rabbit, it produced no definite changes (Kanaar, 1971). Tested at 7% in petrolatum it produced no irritation after a 48-hr closed-patch test on human subjects (Epstein, 1976).

When stearic acid (1·0 M in propanol) was applied daily under occlusive patch tests on human skin, no irritation was produced in 10 days, but intradermal injection of 0·01 or 0·1 M-stearic acid in olive oil produced mild erythema and slight induration in the guinea-pig, rabbit and man (Stillman, Maibach & Shalita, 1975).

Sensitization. A maximization test (Kligman, 1966; Kligman & Epstein, 1975) was carried out on 26 volunteers. The material (RIFM no. 76-253) was tested at a concentration of 7% in petrolatum and produced no sensitization reactions (Epstein, 1976).

Pharmacology. Stearic acid (dose and route not specified) exhibited positive inotropic and positive chronotropic effects on open-chest rabbit-heart preparations and on isolated rabbit-heart preparations (Chopde, Brahamankar & Dorle, 1974).

Cytotoxicity. When 0·2 ml 5% stearic acid in olive oil was administered ip to normal mice, mitosis was inhibited in the intestinal and tongue epithelium (Gyergyay & Gyergyay-Malatinszky, 1966).

Anticarcinogenic effects. Stearic acid (5% in olive oil) administered ip inhibited the *in vivo* mitotic

activity of Ehrlich ascites carcinoma cells in infected mice and decreased the number of polynuclear cells in the tumour tissue (Gyergyay & Gyergyay-Malatinszky, 1966). Stearic acid, at a concentration of 7 mM, provided partial protection to mice when mixed with 6C3HED lymphosarcoma cells or TA_3 mammary carcinoma cells prior to inoculation and gave complete protection when mixed with Ehrlich ascites carcinoma cells (Tolnai & Morgan, 1962). In another study, stearic acid was ineffective when tested for antitumour activity against Ehrlich ascites tumour in mice at 400 mg/kg/day for 5 days; there was one death in six treated animals (Nishikawa, Okabe, Yoshimoto, Kurono & Fukuoka, 1976). When added to a culture of Ehrlich cancer cells at a concentration of 400 μg/ml, it caused inhibition of growth and destruction of cells, but at the other concentrations tested (200, 100, 50 and 25 μg/ml), there was no inhibition of cancer-cell growth (Okada, Nakayama & Mitsui, 1974).

Carcinogenicity assays. Stearic acid, administered 1–3 times a week sc in the inguinal area of female Swiss or BALB/c mice at doses of 0·05–1·0 mg in tricaprylin for 3–57 wk, was not considered carcinogenic (Swern, Wieder, McDonough, Meranze & Shimkin, 1970). The production of subcutaneous sarcomas in BALB/c mice at the lowest dosage was unexpected and unexplained. After a single application of 0·3% 9,10-dimethyl-1,2-benzanthracene in liquid paraffin, stearic acid or a mixture of stearic and palmitic acids applied to the skin of mice 6 times/wk for 31 wk as a 20% solution in chloroform did not promote tumour production (Holsti, 1959). Stearic acid produced no sarcomas when injected sc into mice weekly for 26 wk at levels of 0·5 or 0·05 mg (Van Duuren, Katz, Shimkin, Swern & Wieder, 1972). The acid gave inconclusive results when tested as a co-carcinogen by simultaneous application with benzo[*a*]pyrene to the skin of mice 3 times/wk. After 440 days, the number of animals with tumours and total papillomas was higher than that in control groups whereas the number of animals with carcinomas was not (Van Duuren & Goldschmidt, 1976).

Metabolism. The metabolic pathways in which stearic acid participates, in common with other saturated fatty acids containing an even number of carbon atoms, are reviewed and summarized by White, Handler & Smith (1973). They include energy-producing β-oxidation of the coenzyme (CoA) ester to palmitoyl CoA and acetyl CoA in mitochondria, chain elongation of stearyl CoA to the CoA derivative of the C_{20} acid, arachidic acid, in microsomes, and aerobic desaturation of stearyl CoA to the CoA derivative of oleic acid (C_{18}, one double bond) in microsomes. Stearyl CoA is incorporated into phosphatidic acid, which is then converted to triglyceride in liver and adipose tissue. The free fatty acid may be incorporated into triglyceride in the intestinal mucosa. Phosphoglycerides are intermediate in the production of cholesterol esters from free fatty acid, and stearic acid has participated in this pathway. Phosphoglycerides containing stearic acid residues are produced in cell membranes, the myelin sheath of nerves, and chylomicrons. Stearic acid residues have also been incorporated in brain tissues as cerebrosides.

[^{14}C]Stearic acid, as a human serum albumin complex, was infused into human subjects at rates of 0·4 μCi/min for 30 min during rest and, after an initial 10-min exercise period, for 30 min during exercise (Hagenfeldt & Wahren, 1975). The arterial concentration of stearic acid was lower during exercise than at rest; its turnover was 55% higher than at rest (Hagenfeldt, 1975). The rate of leg uptake increased about threefold while the rate of release from the legs doubled during exercise. The rate of splanchnic uptake decreased during exercise to about half the resting value, although the rate of release was unchanged. The authors state that the reason for the excessive transport of stearic acid during exercise remains to be established. They also state that when [^{14}C]stearic acid is administered iv to rats, the main part of the label is recovered in liver phospholipids.

The oxidation desaturation of stearic acid to oleic acid has been demonstrated in animal-liver microsomes and, more recently, in microsomes isolated from human liver biopsies obtained during operations (De Gomez Dumm & Brenner, 1975). Desaturation activity was low compared to that observed in other mammals, perhaps because of the ages of the patients involved (all adults), preoperative fasting and medication, or the anaesthetic used.

In a review article (*Nutrition Reviews*, 1969), it was stated that fat absorption could be predicted from fatty acid composition, and that unsaturated or short-chain fatty acids favoured absorption while long-chain fatty acids impaired it. Less stearic acid and more linoleic acid favoured fat absorption. Fatty acid in the form of a 2-monoglyceride was better absorbed than the free fatty acid. Stearic acid was the most poorly absorbed of the common fatty acids. Addition of 1 g stearic acid/kg to the diet of lactating cows decreased the absorption of volatile fatty acids into the blood of the portal vein, but did not change the metabolism of these acids by the liver. Liver utilization of ketone bodies in portal blood was decreased and glucose in hepatic blood was increased (Sorokin & Aliev, 1974a,c). The release of low-molecular-weight fatty acids from the wall of the digestive tract was decreased, while utilization of glucose by the wall of the digestive tract and the mammary gland was increased (Sorokin, 1973; Sorokin & Aliev, 1974b). The milk yield was decreased, but the fat content of the milk increased slightly 2 days after supplementation (Martyushov & Aliev, 1974).

In sheep, pancreatic juice and bile apparently facilitated the absorption of stearic acid by the release of oleic and linoleic acids when the secretions mixed (Leat, 1965). [^{14}C]Stearic acid introduced into the duodenum of sheep was incorporated for transport into lymph triglycerides (Leat & Harrison, 1974). It exhibited low rates of turnover and oxidation compared to those obtained for palmitic

acid in starved sheep following iv or intraruminal infusion (Annison, Brown, Leng, Lindsay & West, 1967). Increasing the chain length slightly decreased the digestibility of fatty acids added to the diet of sheep as a 5% (w/w) supplement in the basal ration. Stearic acid was less digestible than lauric, myristic or palmitic acids (Andrews & Lewis, 1970).

Stearic acid fed to chicks was absorbed to the extent of 14%, but when stearic and palmitic acids were fed together, stearic acid absorption was reduced to 2%, and when oleic acid was added to the palmitic and stearic acid diet, stearic acid absorption was increased to 49% (Young & Garrett, 1963). Mixtures of linoleic and oleic acid also enhanced the absorption of stearic acid. Other studies note that stearic acid added to the diet of cockerels was poorly utilized (Koh & Tasaki, 1975a), although body-weight gain, liver size and liver components were not affected. Plasma stearic acid was slightly increased (Koh & Tasaki, 1975b), as was the level in adipose tissue. Lipid levels in the muscle were not affected (Koh & Tasaki, 1975c). Stearic acid tended to accumulate in the yolks of 20-day-old chick embryos, which utilized polyunsaturated fatty acids preferentially (Isaacks, Davies, Ferguson, Reiser & Couch, 1964).

In frogs (*Rana esculenta*) fed stearic–palmitic acid mixtures for 2 days after being starved for several weeks, lipids could not be detected in the gastric mucosa on histological examination (Dominas & Niemierko, 1961). The results of this study indicated that only unsaturated fatty acids are absorbed in the stomach of the frog, and that alkaline phosphatase and certain esterases might be involved.

Radioactively-labelled stearic acid readily entered the lymph from the intestinal tract of the rat (Sieber, Cohn & Wynn, 1974). The incorporation of stearic acid into chylomicron cholesterol ester and triglyceride fractions has been studied by administering mixtures of ^{14}C-labelled palmitic, stearic, oleic and linoleic acids to rats with cannulated thoracic ducts (Karmen, Whyte & Goodman, 1963). The acid mixtures, together with unlabelled fatty acids, were given by gastric intubation. Stearic acid was somewhat less well absorbed and incorporated into both fractions than were the other fatty acids. In a similar study, stearic acid was more readily incorporated into the chylomicron lecithin fraction than the other fatty acids administered (Whyte, Karmen & Goodman, 1963). In rats killed 1 hr after receiving [^{14}C]stearic acid in a 5% fat diet, analysis showed that a high proportion of labelled fatty acid was rapidly incorporated into the phospholipids of the mucosa and muscles. When the experiment was repeated with a 25% fat diet, the triglyceride fraction of the whole mucosa was the most highly labelled (Raghavan, Juneja, Murthy & Ganguly, 1965).

Serum cholesterol increased by approximately 17% in rats receiving a duodenal injection of 300 mg stearic acid in 0·75 ml mineral oil followed by hypothalamic stimulation, a much higher increase than that produced when linoleic or oleic acids or mineral oil were substituted for stearic acid (Gutstein & Farrell, 1972). [^{14}C]Stearic acid, injected as the albumin complex, was extensively desaturated to oleic acid in the livers of carbohydrate re-fed, but not fasted, rats (Elovson, 1965).

References

Andrews, R. J. & Lewis, D. (1970). Utilization of dietary fats by ruminants. II. Effect of fatty acid chain length and unsaturation on digestibility. *J. agric. Sci., Camb.* **75**, part 1, 55.

Annison, E. F., Brown, R. E., Leng, R. A., Lindsay, D. B. & West, C. E. (1967). Rates of entry and oxidation of acetate, glucose, D(−)-β-hydroxybutyrate, palmitate, oleate and stearate, and rates of production and oxidation of propionate and butyrate in fed and starved sheep. *Biochem. J.* **104**, 135.

Nutrition Reviews (1969). Glyceride structure and fat absorption. *ibid* **27**, 18.

Arctander, S. (1969). *Perfume and Flavor Chemicals (Aroma Chemicals)*. Vol. 2, no. 2863. S. Arctander, Montclair, NJ.

Chopde, C. T., Brahamankar, D. M. & Dorle, A. K. (1974). Cardiac stimulant action of fatty acids in mammalian heart. *Indian J. Physiol. Pharmac.* **18**(3), 178.

Connor, W. E., Hoak, J. C. & Warner, E. D. (1963). Massive thrombosis produced by fatty acid infusion. *J. clin. Invest.* **42**, 860.

Council of Europe (1974). Natural Flavouring Substances, Their Sources, and Added Artificial Flavouring Substances. Partial Agreement in the Social and Public Health Field. List 1, no. 15, p. 127. Strasbourg.

De Gomez Dumm, I. N. T. & Brenner, R. R. (1975). Oxidative desaturation of α-linolenic, linoleic, and stearic acids by human liver microsomes. *Lipids* **10**, 315.

Deichmann, W. B., Radomski, J. L., Macdonald, W. E., Kascht, R. L. & Erdmann, R. L. (1958) The chronic toxicity of octadecylamine. *A.M.A. Archs ind. Hlth* **18**, 483.

Dickie, N., Robinson, M. I. & Tuba, J. (1955). The role of alkaline phosphatase in intestinal absorption. III. The effects of various fatty acids on levels of the enzyme in intestinal mucosa. *Can. J. Biochem. Physiol.* **33** (1), 83.

Dominas, H. & Niemierko, W. (1961). Gastric and intestinal fat absorption in the frog. Proceedings of the 6th International Conference on the Biochemistry of Lipids, Marseille, 1960. p. 149.

Elovson, J. (1965). Conversions of palmitic and stearic acid in the intact rat. *Biochim. biophys. Acta* **106**, 291.

Epstein, W. L. (1976). Report to RIFM, 26 April.

Flavoring Extract Manufacturers' Association (1965). Survey of flavoring ingredient usage levels. No. 3035. *Fd Technol., Champaign* **19** (2), part 2, 155.

Food Chemicals Codex (1972). 2nd Ed. Prepared by the Committee on Specifications, Food Chemicals Codex, of the Committee on Food Protection. p. 796. National Academy of Sciences–National Research Council Publ. 1406, Washington, DC.

Gautheron, P. & Renaud, S. (1972). Hyperlipemia induced hypercoagulable state in rat. Role of an increased activity of platelet phosphatidyl serine in response to certain dietary fatty acids. *Thrombosis Res.* **1**, 353.
Gutstein, W. H. & Farrell, G. (1972). Serum cholesterol responses to hypothalamic stimulation and fatty acid administration in the rat. *Proc. Soc. exp. Biol. Med.* **141**, 137.
Gyergyay, F. u. Gyergyay-Malatinszky, E. (1966). Über den Einfluss der Fettsäuren und einiger Öle auf die Vermehrung normaler und tumoraler Epithelzellen. Histochim. Cytochim. Lipides, 5th Simp. Int. Histol., Sofia, 1963. p. 591.
Hagenfeldt, L. (1975). Turnover of individual free fatty acids in man. *Fedn Proc. Fedn Am. Socs exp. Biol.* **34**, 2246.
Hagenfeldt, L. & Wahren, J. (1975). Turnover of plasma free stearic and oleic acids in resting and exercising human subjects. *Metabolism* **24**, 1299.
Hardegree, M. C. & Kirschstein, R. (1968). The toxicity of free fatty acids and Arlacel A. *Ann. Allergy* **26**, 259.
Hoak, J. C., Connor, W. E., Eckstein, J. W. & Warner, E. D. (1964). Fatty acid-induced thrombosis and death: mechanisms and prevention. *J. Lab. clin. Med.* **63**, 791.
Hoak, J. C., Connor, W. E. & Warner, E. D. (1966). Thrombogenic effects of albumin-bound fatty acids. *Archs Path.* **81**, 136.
Holsti, P. (1959). Tumor promoting effects of some long chain fatty acids in experimental skin carcinogenesis in the mouse. *Acta path. microbiol. scand.* **46**, 51.
Isaacks, R. E., Davies, R. E., Ferguson, T. M., Reiser, R. & Couch, J. R. (1964). Avian fat composition. I. Effect of dietary fat on the fatty acids of the triglyceride and phospholipid fractions of the blood plasma and adipose tissue lipids of the laying hen. *Poult. Sci.* **43**, 105.
Janssen, E. T. (1958). Effects of dietary free C15 saturated and unsaturated fatty acids and DL-methionine-choline chloride mixture on plasma cholesterol of the male albino rat. *Proc. Iowa Acad. Sci.* **65**, 234.
Kanaar, P. (1971). Follicular-keratogenic properties of fatty acids in the external ear canal of the rabbit. *Dermatologica* **142**, 14.
Karmen, A., Whyte, M. & Goodman, D. S. (1963). Fatty acid esterification and chylomicron formation during fat absorption. I. Triglycerides and cholesterol esters. *J. Lipid Res.* **4**, 312.
Kligman, A. M. (1966). The identification of contact allergens by human assay. III. The maximization test. A procedure for screening and rating contact sensitizers. *J. invest. Derm.* **47**, 393.
Kligman, A. M. & Epstein, W. (1975). Updating the maximization test for identifying contact allergens. *Contact Dermatitis* **1**, 231.
Koh, T. S. & Tasaki, I. (1975a). Energetic utilization of glucose and saturated fatty acids in adult cockerels. *Nippon Chikusan Gakkai-Ho* **46**, 367.
Koh, T. S. & Tasaki, I. (1975b). Fatty acid distribution of liver and plasma lipids in chicks fed various long-chain fatty acids. *Nippon Chikusan Gakkai-Ho* **46**, 326.
Koh, T. S. & Tasaki, I. (1975c). Effect of dietary fatty acids on fatty acid distribution of lipids in neck adipose pads and superficial pectoral muscle of chicks. *Nippon Chikusan Gakkai-Ho* **46**, 509.
Komarova, E. N. (1976). Toxic properties of some additives for plastics. *Plast. Massy* no. 12, p. 30.
Kritchevsky, D. & Tepper, S. A. (1961). Cholesterol vehicle in experimental atherosclerosis; effect of heated fat. Metab. Parietis Vasorum, Papers 5th Intern. Congr. Angiol., Prague. p. 180.
Leat, W. M. F. (1965). Possible function of bile and pancreatic juice in fat absorption in the ruminant. *Biochem. J.* **94**, 21P.
Leat, W. M. F. & Harrison, F. A. (1974). Origin and formation of lymph lipids in the sheep. *Q. Jl exp. Physiol.* **59**, 131.
Martyushov, V. M. & Aliev, A. A. (1974). Effect of dietary lipids on their absorption by the mammary gland and secretion with milk in cows. In *Lipidny Obmen S-kh. Zhivotn., Sb. Dokl. Vses. Simp., 1st, 1972.* Edited by N. A. Shmanenkov and A. A. Aliev. p. 110. Vses. Nauchno-Issled. Inst. Fiziol., Biokhim. Pitan. S-kh. Zhivotn, Borovsk, USSR.
Merck Index (1976). *An Encyclopedia of Chemicals and Drugs.* 9th Ed. No. 8582. Merck & Co., Inc., Rahway, NJ.
Moreno, O. M. (1976). Report to RIFM, 13 May.
Nishikawa, Y., Okabe, M., Yoshimoto, K., Kurono, G. & Fukuoka, F. (1976). Chemical and biochemical studies on carbohydrate esters. II. Antitumor activity of saturated fatty acids and their ester derivatives against Ehrlich ascites carcinoma. *Chem. pharm. Bull., Tokyo* **24**, 387.
Okada, T., Nakayama, M. & Mitsui, R. (1974). A new approach to the development of anti-cancer agents—5-FU derivatives. *Hiroshima J. med. Sci.* **23**, 51.
Orö, L. & Wretlind, A. (1961). Pharmacological effects of fatty acids, triolein and cottonseed oil. *Acta pharmac. tox.* **18**, 141.
Osumi, Y., Amano, Y., Okegawa, T. & Shimamoto, K. (1966). Effects of ethyl-linoleate on the atheromatous changes caused by high cholesterol diet in the rabbit. *Jap. J. Pharmac.* **16**, 83.
Popjak, G., Holloway, P. W., Bond, R. P. M. & Roberts, M. (1969). Analogues of geranyl pyrophosphate as inhibitors of prenyltransferase. *Biochem. J.* **111**, 333.
Raghavan, S. S., Juneja, H. S., Murthy, S. K. & Ganguly, J. (1965). Uptake, during absorption, of free fatty acids by phospholipids of the intestinal mucosa of rats. *Nature, Lond.* **206**, 189.
Renaud, S. (1968). Thrombogenicity and atherogenicity of dietary fatty acids in rat. *J. Atheroscler. Res.* **8**, 625.
Renaud, S. (1969). Thrombotic, atherosclerotic and lipemic effects of dietary fats in the rat. *Angiology* **20**, 657.
Sieber, S. M., Cohn, V. H. & Wynn, W. T. (1974). Entry of foreign compounds into the thoracic duct lymph of the rat. *Xenobiotica* **4**, 265.
Sorokin, V. M. (1973). Effect of saturated and unsaturated fatty acids in the ration on the volatile fatty acid, glucose and ketone body metabolism in the digestive tract wall and the mammary gland of cows. In *Fiziol.-Biokhim. Genet. Osn. Povysh. Eff. Ispol'z. Kormov. Zhivotnovod., Tezisy Dokl. Vses. Soveshch. 1973.*

Edited by N. A. Shmanenkov. Vol. I, p. 93. Vses. Naucho-Issled. Inst. Fiziol., Biokhim. Pitan. S-kh. Zhivotn. Borovsk, USSR.

Sorokin, V. M. & Aliev, A. A. (1974a). Effect of saturated and unsaturated fatty acids in rations on indexes of the carbohydrate-lipid metabolism in cows. In *Lipidnyi Obmen S-kh. Zhivotn., Sb. Dokl. Vses. Simp., 1st, 1972.* Edited by N. A. Shmanenkov and A. A. Aliev. p. 181. Nauchno-Issled. Inst. Fiziol., Biokhim. Pitan. S-kh. Zhivotn., Borovsk, USSR.

Sorokin, V. M. & Aliev, A. A. (1974b). Effect of fatty additives on metabolism in lactating cows. I. Carbohydrate-lipid metabolism in the digestive-tract wall of cows with single loadings of their ration with stearic and palmitic acids. *Byull. Vses. Nauchno-Issled. Inst. Fiziol., Biokhim. Pitan. S-kh. Zhivotn.* **8** (3), 55.

Sorokin, V. M. & Aliev, A. A. (1974c). Effect of fatty additives on metabolism in lactating cows. II. Carbohydrate-lipid metabolism in the liver of lactating cows with single loads of their ration with saturated fatty acids. *Byull. Vses. Nauchno-Issled. Inst. Fiziol., Biokhim. Pitan. S-kh. Zhivotn.* **8** (4), 53.

Stillman, M. A., Maibach, H. I. & Shalita, A. R. (1975). Relative irritancy of free fatty acids of different chain length. *Contact Dermatitis* **1**, 65.

Sunde, M. L. (1956). The effect of fats and fatty acids in chick rations. *Poult. Sci.* **35**, 362.

Swern, D., Wieder, R., McDonough, M., Meranze, D. R. & Shimkin, M. B. (1970). Investigation of fatty acids and derivatives for carcinogenic activity. *Cancer Res.* **30**, 1037.

Tachikawa, M. (1974). Effects of plasma lipids on blood coagulation. *Niigata Igakkai Zasshi* **88** (6), 269.

Tolnai, S. & Morgan, J. F. (1962). Studies on the *in vitro* antitumor activity of fatty acids. VI. Derivatives of mono- and di-carboxylic and unsaturated fatty acids. *Can. J. Biochem. Physiol.* **40**, 1367.

United States Pharmacopeia (1975). 19th Revision. Prepared by the Committee of Revision. p. 575. The United States Pharmacopeial Convention, Inc., Washington, DC.

Van Duuren, B. L., Katz, C., Shimkin, M. B., Swern, D. & Wieder, R. (1972). Replication of low-level carcinogenic activity bioassays. *Cancer Res.* **32**, 880.

Van Duuren, B. L. & Goldschmidt, B. M. (1976). Cocarcinogenic and tumor-promoting agents in tobacco carcinogenesis. *J. natn. Cancer Inst.* **56**, 1237.

White, A., Handler, P. & Smith, E. L. (1973). *Principles of Biochemistry.* 5th Ed. pp. 542 & 579. McGraw-Hill, New York.

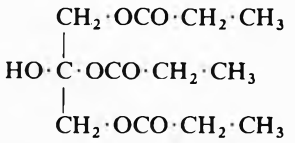
Whyte, M., Karmen, A. M. & Goodman, D. S. (1963). Fatty acid esterification and chylomicron formation during fat absorption: 2. Phospholipids. *J. Lipid Res.* **4**, 322.

Young, R. J. & Garrett, R. L. (1963). Effect of oleic and linoleic acids on the absorption of saturated fatty acids in the chick. *J. Nutr.* **81**, 321.

TRIETHYL CITRATE

Synonym: Ethyl citrate.

Structure:



Description and physical properties: *Merck Index* (1976).
Occurrence: Has apparently not been reported to occur in nature.
Preparation: By esterification of ethyl alcohol with citric acid (Arctander, 1969).
Uses: Concentration in final product (%):

	Soap	*Detergent*	*Creams, lotions*	*Perfume*
Usual	0·03	0·015	0·015	0·5
Maximum	0·5	0·05	0·15	3·0

Status

Triethyl citrate was given GRAS status by FEMA (1965), is approved by the FDA for food use (GRAS) and was included by the Council of Europe in the list of artificial flavouring substances not fully evaluated. The *Food Chemicals Codex* (1972) has a monograph on triethyl citrate. CAS Registry No. 77–93–0.

Biological data

Acute toxicity. The oral LD_{50} of triethyl citrate in rats was approximately 7 ml/kg; toxic signs included weakness, depression, ataxia, hyperexcitability, unrest, urinary dribbling, irregular and laboured respiration and convulsions (Finkelstein & Gold, 1959). The oral LD_{50} in cats was approximately 3·5 ml/kg; toxic signs included nausea, vomiting, ataxia, weakness, muscle twitching, tremors, reflex hyperexcitability, a lowering of body temperature, gasping and shallow respiration, prostration, convulsions and respiratory failure (Finkelstein & Gold, 1959). The ip LD_{50} in mice was 1·75 g/kg, death being attributed to circulatory collapse and postictal depression (Meyers, Autian & Guess, 1964). The dermal LD_{50} for triethyl citrate in the guinea-pig was >10 ml/kg (Fassett, 1963), while that in rabbits has been reported as >5 g/kg (Levenstein, 1975).

Behavioural toxicity. Triethyl citrate administered ip to mice in a dose of 400 mg/kg produced loss of righting reflex without loss of consciousness (Meyers *et al.* 1964). Posture was regained within 15 min. Respiration was increased and frequently clonic convulsions were observed. Ip administration of 400 mg/kg to rats and iv administration to rabbits confirmed the stimulating properties of triethyl citrate. A dose of 1 g/kg placed in the ventral lymph sac of the frog resulted in clonic activity and the loss of all reflex activity (Meyers *et al.* 1964).

Subacute toxicity. In mice given daily ip doses of 350 mg triethyl citrate/kg for 14 days, normal weight gain was inhibited, but there were no changes in blood chemistry and no histological changes (Meyers *et al.* 1964). Rats given triethyl citrate in the diet at concentrations of 0·5, 1·0 and 2% for 6 or 8 wk showed no effects on growth or blood chemistry, and no gross or histological changes (Finkelstein & Gold, 1959). Fed at 5% in the diet of rats for 12 days, the ester caused one death in eight rats and decreased body weight (Yoshida, Ikumo & Suzuki, 1971). It was an unexpectedly poor energy source in the diet of chicks (Yoshida, Morimoto & Oda, 1970).

In cats given 0·25 ml triethyl citrate/kg/day orally for 8 wk, no changes in weight or blood chemistry were found, and electrocardiograms did not differ from controls (Finkelstein & Gold, 1959). After four or five doses, mild toxic signs were present but all animals survived the treatment period and recovered when treatment was discontinued, although cumulative toxic effects were reported (Finkelstein & Gold, 1959).

Inhalation. With triethyl citrate vapour, the LC_{50} for a 6-hr exposure of rats was 1300–3500 ppm, with the possibility that some decomposition occurred during vaporization (Fassett, 1963). Rats tolerated 6-hr daily exposures of 296 ppm for 62 days. At higher concentrations, reactions included gasping and weakness, and pleural infusion and pulmonary oedema occurred (Fassett, 1963).

Irritation. Triethyl citrate applied full strength to intact or abraded rabbit skin for 24 hr under occlusion was not irritating (Levenstein, 1975). Tested at 20% in petrolatum, it produced no irritation after a 48-hr closed-patch test on human subjects (Epstein, 1975).

Sensitization. A maximization test (Kligman, 1966; Kligman & Epstein, 1975) was carried out on 22 volunteers. The material (RIFM no. 74–250) was tested at a concentration of 20% in petrolatum and produced no sensitization reactions (Epstein, 1975).

Metabolism. Rat-, mouse- and human-liver homogenates as well as serum enzymes cleaved triethyl citrate to 1 mol citric acid and 3 mol ethanol/mol ester (Bruns & Werners, 1962).

Pharmacology. Triethyl citrate blocked nerve conduction in the rat and produced cord depression, temporarily abolished the corneal reflex in the rabbit eye, exhibited local anaesthetic activity in the guinea-pig, and decreased blood pressure in rabbits and cats, causing smooth-muscle depression or cardiac depression (Meyers *et al.* 1964).

Cytotoxicity. Triethyl citrate in concentrations of 3–12 $\times 10^{-3}$ M inhibited the growth of strain L mouse fibroblasts. The toxicity was inherent in the intact molecule, and hydrolysis to citric acid and chelation of Ca^{2+} ion by citrate were ruled out as possible explanations of toxicity (Rosenbluth, Guess & Autian, 1967). The mechanism of growth inhibition appeared to be initial mitochondrial involvement, with secondary DNA repression (Golaz, Guess & Autian, 1967). Some experimental evidence indicated protein denaturation as the reason for toxicity, especially at a concentration of 12 mM, together with a decreased production of ATP at concentrations below 12 mM (Jones, Guess & Autian, 1968).

References

Arctander, S. (1969). *Perfume and Flavor Chemicals (Aroma Chemicals)*. Vol. 2, no. 2990. S. Arctander, Montclair, NJ.

Bruns, F. H. u. Werners, H. P. (1962). Zum Stoffwechsel von Triäthylcitrat und Acetyltriäthylcitrat. *Klin. Wschr.* **40**, 1169.

Council of Europe (1974). Natural Flavouring Substances, Their Sources, and Added Artificial Flavouring Substances. Partial Agreement in the Social and Public Health Field. List 3, no. 4093, p. 368. Strasbourg.

Epstein, W. L. (1975). Report to RIFM, 15 August.

Fassett, D. W. (1963). Esters. In *Industrial Hygiene and Toxicology*. 2nd Ed. Edited by F. A. Patty. Vol. II, p. 1892. Interscience Publishers, New York.

Finkelstein, M. & Gold, H. (1959). Toxicology of the citric acid esters: tributyl citrate, acetyl tributyl citrate, triethyl citrate, and acetyl triethyl citrate. *Toxic. appl. Pharmac.* **1**, 283.

Flavoring Extract Manufacturers' Association (1965). Survey of flavoring ingredient usage levels. No. 3083. *Fd Technol., Champaign* **19** (2), part 2, 155.

Food Chemicals Codex (1972). 2nd Ed. Prepared by the Committee on Specifications, Food Chemicals Codex, of the Committee on Food Protection. p. 837. National Academy of Sciences–National Research Council Publ. 1406, Washington, DC.

Golaz, M., Guess, W. L. & Autian, J. (1967). Mechanistic toxicology of triethyl citrate in mouse fibroblast cells by liquid scintillation techniques. *J. pharm. Sci.* **56**, 1252.

Jones, A. B., Guess, W. L. & Autian, J. (1968). Mechanistic toxicology of triethyl citrate in cultured mammalian cells by cinematography. *J. pharm. Sci.* **57**, 293.

Kligman, A. M. (1966). The identification of contact allergens by human assay. III. The maximization test. A procedure for screening and rating contact sensitizers. *J. invest. Derm.* **47**, 393.

Kligman, A. M. & Epstein, W. (1975). Updating the maximization test for identifying contact allergens. *Contact Dermatitis* **1**, 231.

Levenstein, I. (1975). Report to RIFM, 30 May.

Merck Index (1976). *An Encyclopedia of Chemicals and Drugs*. 9th Ed. No. 3719. Merck & Co., Inc., Rahway, NJ.

Meyers, D. B., Autian, J. & Guess, W. L. (1964). Toxicity of plastics used in medical practice. II. *J. pharm. Sci.* **53**, 774.

Rosenbluth, S. A., Guess, W. L. & Autian, J. (1967). Growth changes in mammalian cell cultures by plastic additives (effect of triethyl citrate). *J. biomed. Mater. Res.* **1**, 197.

Yoshida, M., Ikumo, H. & Suzuki, O. (1971). Evaluation of available energy of aliphatic chemicals by rats. An application of bioassay of energy to mono-gastric animal. *Agric. biol. Chem.* **35**, 1208.

Yoshida, M., Morimoto, H. & Oda, R. (1970). Availability of energy in esters of aliphatic acids and alcohols by growing chicks. *Agric. biol. Chem.* **34**, 1668.

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Review Section

SHORT REVIEW

POSSIBLE USE OF DIETARY SURVEYS TO ASSESS INTAKE OF FOOD ADDITIVES*

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(*Received 11 October 1978*)

Introduction

For any additive, the amount permitted for use in foodstuffs needs to be decided in the light of expert opinion on the safe daily intake and on an assessment of the maximum amount likely to be consumed by individuals who have the highest intakes of the foods in which the additive is to be used. This paper is concerned only with the problems of making such an assessment.

Following its meeting in 1970, the FAO/WHO Expert Committee on Food Additives (1971) recommended that where surveys of the consumption of foods by individuals were being planned for nutritional purposes, those responsible should modify the design so that information would be provided on the amounts of various non-nutritive food additives consumed by individuals. This request came too late for changes to be made in the design of the field work of the nutrition survey on which this paper is based, but not too late for additions to be made to the programs for data processing. Although, for reasons to be discussed, the information obtained on additive intake was limited, much methodological experience was gained.

Acquisition and processing of data

From September 1970 to August 1971 a study was made of school children aged 14–15 yr, who were randomly selected from all schools situated in the south-east area of the City of Birmingham. Dietary and anthropometric data were obtained from 764 children—375 boys and 389 girls.

Trained dietary observers supervised the completion of a weighed record of all food and drink ingested over a period of seven consecutive days. A system of cumulative weighing was used. Children who had school meals weighed them in the same way. A record was also kept of any sweets, snacks or drinks consumed between meals so that appropriate weights could be attributed. A medical assessment and anthropometric measurements of each child failed to reveal any evidence of malnutrition except in a small percentage of children (3%) who were classified as obese.

The information obtained was used to assess the intakes of three food additives: sorbic acid, saccharin and benzoic acid. Much of this paper deals with methodological problems common to the assessment of any additive used in food, and where this is so the word 'additive' is used without specification.

The preparation of the Food Composition Table has been described elsewhere (Department of Health and Social Security, 1972; Ministry of Health, 1968). The Table contained nutritional information on 587 separate items of food commonly consumed in the UK and was based on information derived from laboratory analysis or from manufacturers, or calculated from typical recipes used by the British housewife in the preparation of meals. The table was originally designed to collect information about energy and nutrient intakes for the detection of under- or over-nutrition. Such a table of food composition is not the ideal instrument for deriving information about food additives. Foods that may be grouped together for nutrient content (for example, bought and home-made pies and tarts) should be coded separately if distinction is to be made between those containing additives and those without.

The dietary information was re-processed after a figure for each additive had been attributed to each food code. Three main assumptions were made. The first, which applies to all dietary surveys made over a limited period of time, was that the information collected for the week of the survey was representative of the usual diet. The second assumption was that in any particular food, manufacturers had used the maximum amount of the additive permitted. This was not necessarily true since good manufacturing practice demands the use of the minimum amount of additive needed to achieve the required technological effect. Thirdly, where coding did not distinguish between the bought and home-made product, the assumption was made in every case that the food

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Table 1. *Mean daily intakes of benzoic acid, saccharin and sorbic acid by boys and girls aged 14–15 yr*

Additive	ADI (mg/kg body weight)	Group†	Intake‡ Mean	SD	Range
			mg/day		
Benzoic acid		B	34·6	35·0	0–311·0
		G	30·9	29·3	0–216·2
Saccharin		B	13·4*	14·8	0–157·7
		G	10·4	11·7	0–170·0
Sorbic acid		B	66·5*	43·3	0·1–281·1
		G	56·4	36·4	0·1–230·6
			mg/kg body weight/day		
Benzoic acid	0–5§	B	0·64	0·6	0–5·34
		G	0·58	0·6	0–3·73
Saccharin	0–2·5‖	B	0·25*	0·3	0–2·51
		G	0·20	0·2	0–1·84
Sorbic acid	0–25§	B	1·23*	0·8	0·01–5·15
		G	1·08	0·7	0·01–4·74

†Groups of 375 boys (B) or 389 girls (G).
‡Figures calculated from food-consumption records assuming that the additives were present in foods (even those that were, in fact, home-made) at the maximum permitted levels.
§Joint FAO/WHO Expert Committee (1974).
‖Joint FAO/WHO Expert Committee (1978).
Asterisks mark the boys' mean intakes that were significantly higher (*$P < 0{\cdot}01$ by a test between the means) than the corresponding figure for the girls.

eaten was the bought product, and therefore the additive that would be present in the bought product, but not necessarily in the home-made product, was included. These two latter assumptions inevitably resulted in an over-estimate of the intake.

In about 10% of subjects a second study was made and the additive intakes on the two occasions were compared.

Results

Table 1 shows the mean daily intakes of benzoic acid, saccharin and sorbic acid by the boys and by the girls. Most (but not all) of the children ate foods (or drink) containing benzoic acid and saccharin and all of them ingested sorbic acid. Boys ingested, on average, more of each additive than girls, both in absolute terms and in proportion to body weight, although only in the mean intakes of saccharin and sorbic acid were the differences statistically significant. The ranges of individual intakes were large.

Figures 1 and 2 show, for boys and girls, the percentage frequency distribution of additive intakes expressed as a percentage of the acceptable daily intake (ADI), defined as the daily dose of an additive which, during an entire lifetime, appears to be without appreciable risk, on the basis of all the facts known at the time (Lu, 1973). The distributions in each case are skewed to the right. The intakes of sorbic acid show a narrower spread than those of saccharin and benzoic acid. Intakes below 25% of the ADI were recorded for sorbic acid in all subjects, for saccharin in 94% of the children and for benzoic acid in 88%, but two boys had calculated intakes of saccharin approximately equal to the ADI, and one boy exceeded the ADI for benzoic acid by 7%.

Figure 3 shows the foods that contributed to the intake of each of the three additives. Soft drinks accounted for over 50% of the intake of benzoic acid and for about 75% of the intake of saccharin, while cakes, biscuits and pastries accounted for over 80% of the sorbic acid intake.

The 10% of subjects with the highest intakes of the additives were considered separately. In general, these subjects ate the same foods as the others. Their intakes of additives were larger because more of each food was eaten. An exception worth noting was that those with a high intake of saccharin (and to a lesser extent of benzoic acid) consumed, proportionately as well as absolutely, more soft drinks than the remainder of the sample. The 10% of children with the highest intakes of saccharin derived 85% of this intake from soft drinks. The rest of the children obtained only 65% of their saccharin intake from soft drinks. Similarly 60% of the benzoic acid intake of the 10% with the highest intakes of this additive were obtained from soft drinks. For the other 90% of the children, soft drinks provided only 50% of the benzoic acid intake.

In the small group of children studied twice, wide differences were commonly found between the additive intakes on the two occasions.

Discussion

It is reassuring to know that, although they were certainly over-estimates, the calculated intakes of the three additives, during the week of the study, were well below the ADIs for almost all the children.

The main interest of the study, however, is methodological. It ought to be possible to supplement Hansen's logical approach to the problem of statutory

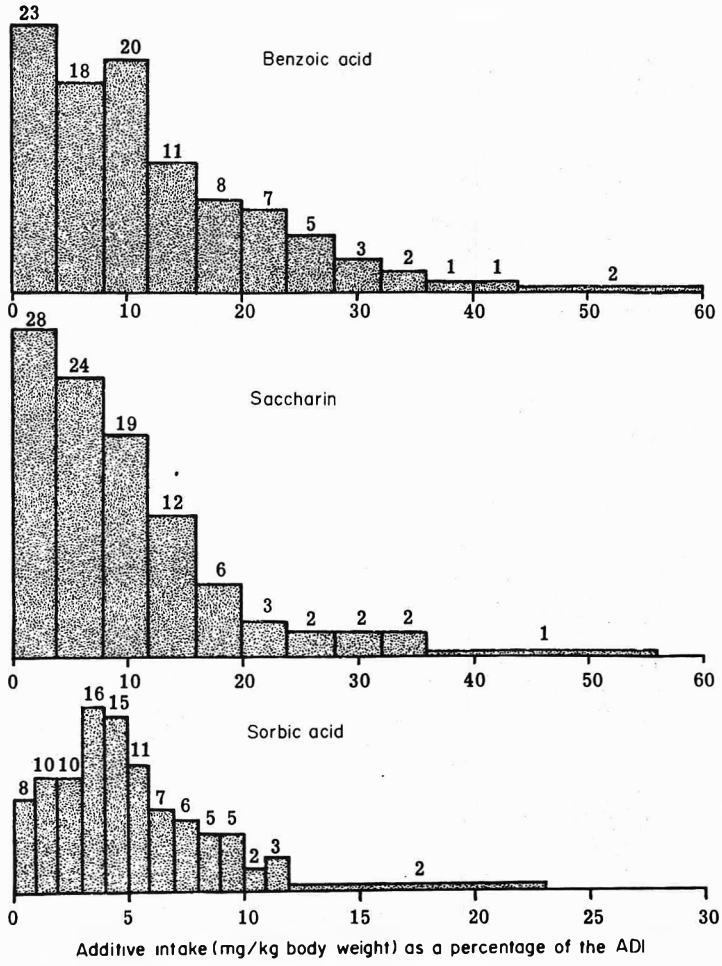

Fig. 1. Percentage frequency distributions of additive intakes (mg/kg body weight) for 375 boys, expressed as a percentage of the acceptable daily intake: (a) benzoic acid (ADI 0–5 mg/kg), (b) saccharin (ADI 0–2·5 mg/kg); (c) sorbic acid (ADI 0–25 mg/kg). For four of the boys a daily benzoic acid intake greater than 60% of the ADI was calculated, assuming tnat the additive was present at the maximum permitted levels in all the foods consumed, whether bought or home-made. Three boys had saccharin intakes greater than 60% of the ADI.

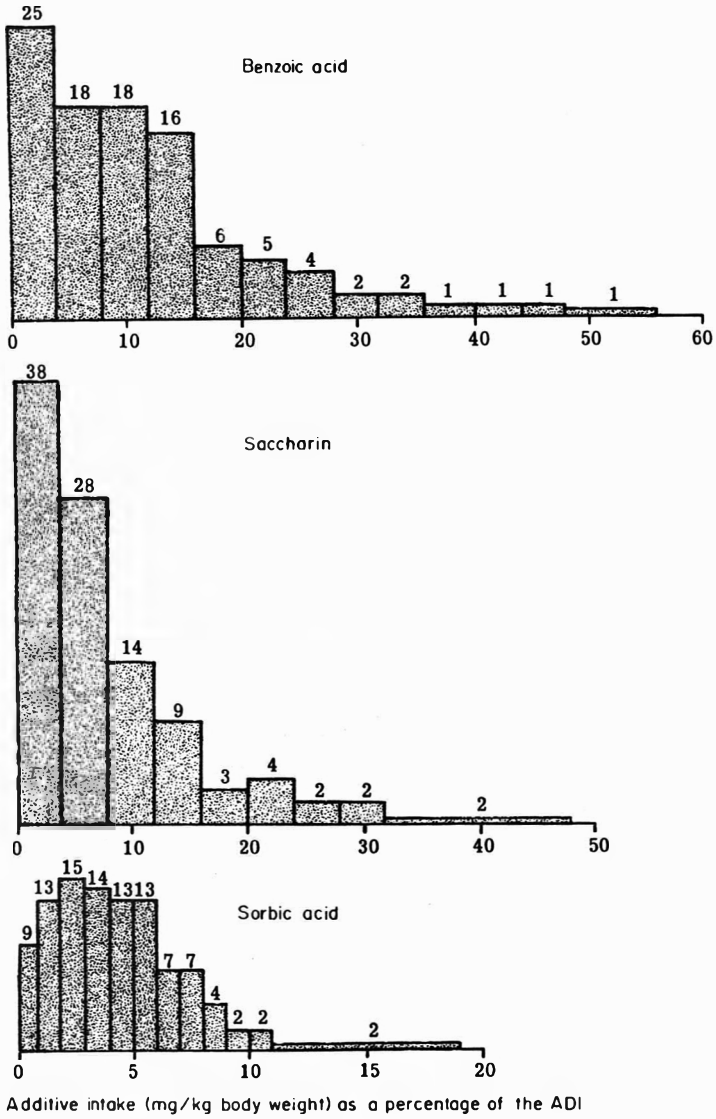

Fig. 2. Percentage frequency distributions of additive intakes (mg/kg body weight) for 389 girls, expressed as a percentage of the acceptable daily intake: (a) benzoic acid (ADI 0-5 mg/kg); (b) saccharin (ADI 0–2·5 mg/kg); sorbic acid (ADI 0–25 mg/kg). For one girl, a daily benzoic acid intake greater than 60% of the ADI was calculated, assuming that the additive was present at the maximum permitted levels in all the foods consumed, whether bought or home-made. There was also one girl with a saccharin intake above 60% of the ADI.

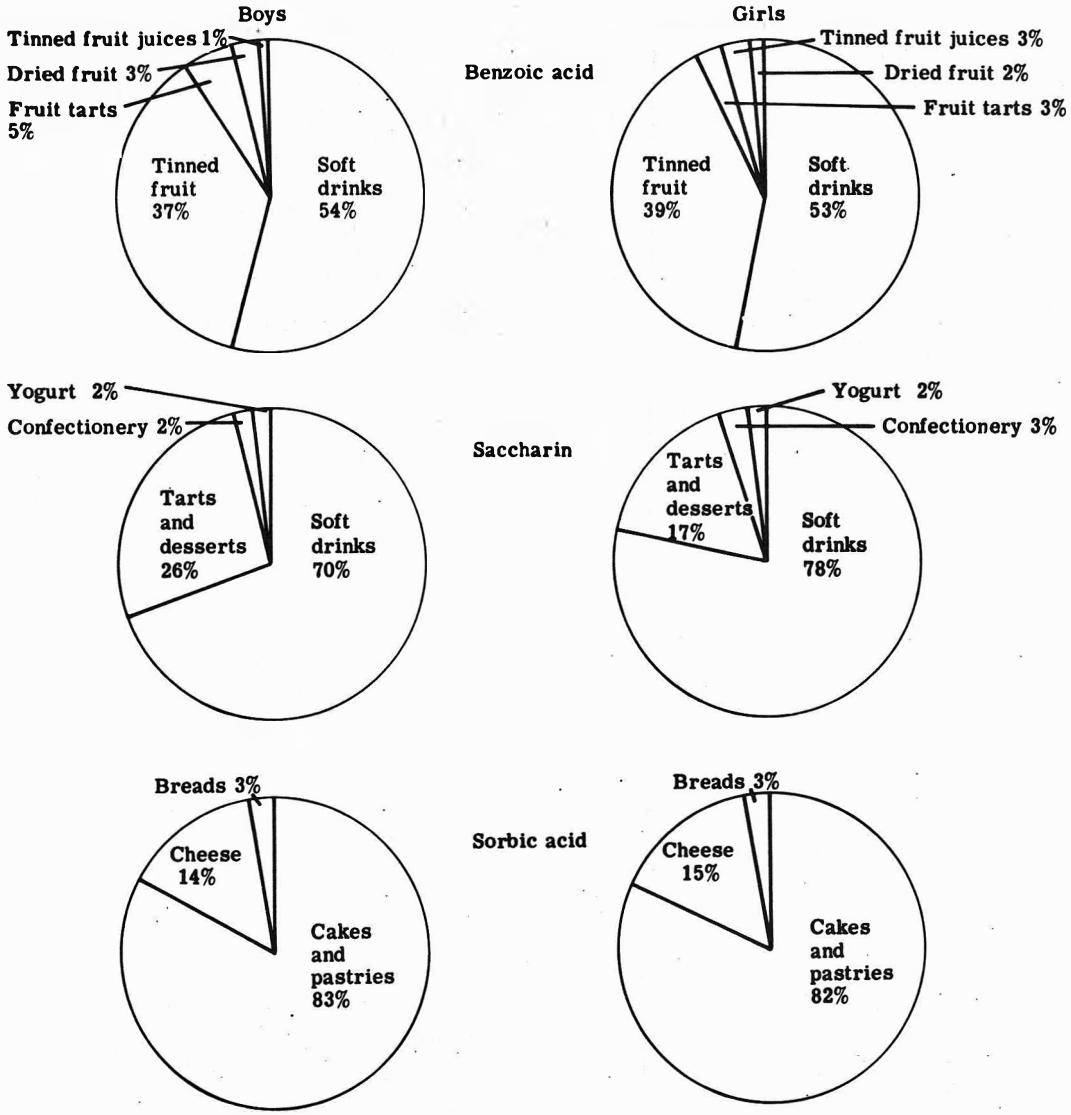

Fig. 3. Proportions of different foods contributing to the mean daily intakes of (a) benzoic acid, (b) saccharin and (c) sorbic acid in 375 boys and 389 girls aged 14–15 yr.

tolerances (Hansen, 1966) with information from dietary surveys on the amounts of additive actually consumed by those with the highest intakes. Some of the problems that remain to be solved are fairly simple, such as the compilation and use of tables giving separately the data for home-made and bought foods. A much harder problem is to devise ways of assessing the effect of time in concentrating the scatter of recorded individual intakes around the mode, in order to arrive at a realistic estimate of the additive intake of the persistently high consumer whose protection is the main aim of statutory tolerances. Nevertheless, we believe from our experience that a practicable, albeit expensive, method can be devised.

REFERENCES

Department of Health and Social Security (1972). A Nutrition Survey of Pre-school Children 1967–68. Report on Health and Social Subjects, No. 10. HMSO, London.

Hansen, S. C. (1966). Acceptable daily intake of food additives and ceiling on levels of use. *Fd Cosmet. Toxicol.* **4**, 427.

Joint FAO/WHO Expert Committee on Food Additives (1971). Fourteenth Report—Evaluation of Food Additives. *Tech. Rep. Ser. Wld Hlth Org.* **462**, p. 26.

Joint FAO/WHO Expert Committee on Food Additives (1974). Seventeenth Report—Toxicological Evaluation of Food Additives with a Review of General Principles and of Specifications. *Tech. Rep. Ser. Wld Hlth Org.* **539**, pp. 16 & 18.

Joint FAO/WHO Expert Committee on Food Additives (1978). Twenty-first Report—Evaluation of Certain Food Additives. *Tech. Rep. Ser. Wld Hlth Org.* **617**, p. 38.

Lu, F. C. (1973). Toxicological evaluation of food additives and pesticide residues and their "acceptable daily intakes" for man. The role of WHO, in conjunction with FAO. In *Residue Reviews. Residues of Pesticides and Other Contaminants in the Total Environment.* Edited by F. A. Gunther. p. 81. Springer-Verlag, New York.

Ministry of Health (1968). A Pilot Survey of the Nutrition of Young Children in 1963. Report on Public Health and Medical Subjects, No. 118. HMSO, London.

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REVIEWS OF RECENT PUBLICATIONS

Evaluation of Certain Food Additives and Contaminants. Twenty-second Report of the Joint FAO/WHO Expert Committee on Food Additives. Tech. Rep. Ser. Wld Hlth Org. 1978, no. 631. pp. 39. Sw.fr. 5.00 (available in the UK through HMSO).

The re-evaluation of a number of food colourings was a major task of the Expert Committee at their latest meeting, held in Rome in April 1978. Previously allocated temporary ADIs (in mg/kg) of up to 0·75 for amaranth, 2·5 for Brilliant Black PN, 0·125 for Ponceau 4R, 0·5 for quinoline yellow, 2·5 for turmeric, 0·1 for curcumin and "not specified" for beet red, iron oxides and hydrated iron oxide were extended and that for azorubine was increased from 0·5 to 1·25, subject in each case to further data becoming available within a specified period. The information required ranged from a study of the absorption and storage of iron from use of iron oxides (needed by 1979) to long-term tests in two species, a short-term test in a non-rodent mammal, multigeneration and teratogenicity studies and metabolic studies in several species for beet red (needed by 1982). The controversial FDA study on amaranth (*Food Chemical News* 1976, **17** (43), 68) was dismissed on the grounds of technical inadequacy but further long-term tests in two species were requested in view of the potentially wide use of this colouring.

The sweeteners sorbitol and xylitol were also re-evaluated. As 20% dietary sorbitol had produced adverse effects in rats and dogs and no lower levels were studied, the previous "ADI not specified" was changed to "temporary ADI not specified". Further data required by 1980 were a long-term rat test directing particular attention to effects on the thyroid and adrenal, a multigeneration study, and a 6-month dog study with emphasis on liver function and histopathology. For xylitol, no ADI could be established, in view of bladder tumours found in male mice and adrenal tumours in rats. The Committee recommended further long-term tests in two rat strains exposed *in utero*, as well as studies of adrenal function in rats and man and urine chemistry in man.

Other additives considered by the Committee included maltol, for which recent data enabled the establishment of a new temporary ADI of up to 0·5 mg/kg, and dioctyl sodium sulphosuccinate, for which the temporary ADI established in 1974 was withdrawn on the grounds that the requested data had not been provided. As sodium thiosulphate decomposes in the stomach to sulphur dioxide and sulphur, it was incorporated in the group ADI of up to 0·7 mg/kg (as SO_2) allocated for sulphur dioxide and sulphites. The use of aluminium metal for silvering confectionery was not considered to present a hazard, but data on aluminium sulphate, aluminium potassium sulphate and aluminium sodium sulphate were insufficient for evaluation. Nitrous oxide used as a food propellant and stannous chloride in its very limited use as a food additive were also deemed to be without hazard.

A number of food contaminants were also reviewed. Although unequivocal evidence relating asbestos ingestion to cancer was still lacking, the strictest precautions against environmental contamination were regarded as necessary. However, monitoring for asbestos in food was not considered feasible at present. For lead and mercury the provisional tolerable weekly intakes established in 1972 were reaffirmed, but available data still did not allow a tolerable lead intake to be recommended for children. No tolerable weekly intake could be established for inorganic tin salts, but in view of extensive human experience it was concluded that the presence of such salts in food could be limited by good manufacturing practice. For organotin compounds it was not possible to establish a tolerable intake for the group, and individual evaluations were considered necessary for the future.

The Committee recognized the urgent need for an inventory of the additives and contaminants not yet evaluated, and recommended that an expert working group should compile such an inventory and establish priorities for testing and evaluation. Compounds of high priority should be tested comprehensively in animals. However, those of lower priority (i.e. those not structurally related to substances of demonstrated toxicity and consumed only in very small quantities) may need to be subjected only to an abbreviated testing programme, guidelines for which should be established by an expert group. Another meeting of experts should be convened to assess the value of feeding studies on the progeny of exposed parents, and to propose guidelines for experimentation.

Toxicological monographs were compiled on amaranth, azorubine, Brilliant Black PN, Ponceau 4R, quinoline yellow, turmeric and curcumin, sorbitol, xylitol, maltol, stannous chloride, asbestos, lead and mercury, and specifications were revised or tentatively prepared for 32 substances, including eight microbial-enzyme preparations and 15 salts. Because of financial stringencies the monographs, entitled *Toxicological Evaluation of Certain Food Additives* (WHO Fd Add. Ser. no. 13), are regrettably being made available only in document form on request to WHO.

Toxicological Evaluation of Certain Food Additives. WHO Fd Add. Ser. 1978, no. 10. pp. 134.

Summary of Toxicological Data of Certain Food Additives. WHO Fd. Add. Ser. 1978, no. 12. pp. 147.

The first of the above documents resulted from the 20th (1976) meeting of the Joint FAO/WHO Expert Committee on Food Additives (*Cited in F.C.T.* 1977,

15, 639) and contains monographs on butylated hydroxyanisole, butylated hydroxytoluene, diethylene glycol monoethyl ether, the dodecyl, octyl and propyl gallates, glycerol and glycerol diacetate, menthol, food-grade mineral oil, natamycin (pimaricin), sodium and potassium nitrites, sucrose esters of fatty acids and sucroglycerides and trichloroethylene. Its publication was regrettably delayed by a shortage of funds, but for the most part this delay has not diminished its value appreciably. Only perhaps in the case of nitrites have subsequent findings (*Food Chemical News*, 1978, **20** (22), 65) led to a need for a basic reappraisal. We hope that it will not seem churlish to add the comment that sufficient finance does not seem to have been provided for a proof reader. to judge from the many printing errors, a totally inappropriate heading of "food colours" on one of the early pages and the need to subtract two from all the page numbers given in the contents list.

The second document, the result of the 21st FAO/WHO meeting (*Cited in F.C.T.* 1978, **16**, 615), contains monographs on adipic acid, tartaric acid and monosodium tartrate, the food colourings aluminium, Benzyl Violet 4B, Black 7984, Brown FK, caramels (ammonia and ammonia-sulphite processes), carthamus yellow, Chocolate Brown FB, Chocolate Brown HT, chrysoine, cochineal, carmine and carminic acid, Orange I, Orange GGN, Orange RN, Ponceau SX, Red 2G, silver and Yellow 2G, and the sweetening agents sodium and potassium cyclamate and xylitol. It is surprising to find that the conclusions reached by the Committee on these additives have been omitted, which means that the volume must always be read in conjunction with the corresponding Technical Report (no. 617, 1978), and again there is evidence of a lack of proof reading. More seriously, since the Committee does not perform its own literature searches but is entirely reliant on data voluntarily submitted to it, the coverage of relevant literature is sometimes far from complete. This is particularly noticeable in the cyclamate monograph, which reviews only a small fraction of the available information on the reproductive and mutagenic effects of cyclohexylamine.

It must be deplored that the Expert Committee, whose impartial pronouncements are of such great value to all those concerned with food additives, should apparently be starved of the means to allow it to function effectively. It is to be hoped that this situation will not be allowed to continue.

Substances Used in Plastics Materials Coming into Contact with Food. P.SG (78) 26. Council of Europe, Strasbourg, 1978. pp. 81.

The first edition of the Council of Europe's official list of plastics materials for use in contact with food is now available free of charge from the Council of Europe offices. Copies may be obtained from Mr. O. Messner, Deputy Director of Economic and Social Affairs, Council of Europe, 67006 Strasbourg Cedex, France, but interested parties should direct their comments on the document to the delegation of the country in which they are based, rather than to Council of Europe officials who have no means of handling the enquiries or comments.

The published list is preceded by an introduction, which defines the objectives of the exercise, and by a preamble describing the toxicological and generic classifications assigned by the Committee of Experts. As in the provisional classification, some substances have been judged to require no restriction other than the contamination limit of 60 mg/kg food, others have been given a specific limitation in food, a few compounds have been rejected and a large number of substances could not be evaluated. For convenience, the Committee of Experts also divided the large number of plastics ingredients into two sections and two appendices. The first section includes monomers and other starting materials and the second contains additives and polymerization aids. Appendix 1 is a temporary list of polymers and Appendix 2 lists substances notified but not yet considered.

The document ends with a set of guidelines on procedures for approving new components in plastics formulations used as food-contact materials. This includes sections on the factors that may affect the degree of health hazard associated with any particular material, the identity of migrants and the extent of toxicological testing required. While a full toxicological assessment of every component is considered essential, the Committee concedes that it may be possible to accept certain substances for a limited period largely on the basis of a sound 90-day study.

In future, the Committee of Experts will review the list annually with a view to effecting any amendments that may be necessary in the light of toxicological and technological developments. Interested parties may submit detailed proposals for such amendments to their national authorities. (In the UK, proposals should be sent to Dr J. R. Bell of the Food Science Division, MAFF).

The Biogeochemistry of Lead in the Environment. Part A. Ecological Cycles. Part B. Biological Effects. Edited by J. O. Nriagu. Elsevier/North-Holland Biomedical Press, Amsterdam, 1978. Part A: pp. xi + 422. Dfl. 165.00. Part B: pp. xi + 397. Dfl. 155.00.

These two volumes mark the start of an ambitious new series by Elsevier on topics in environmental health. They clearly demonstrate the many areas in which lead is important. The individual chapters of both volumes have been contributed by experts in each field.

The first volume covers topics related to the ecological cycling of lead. A brief general chapter on the properties and the global cycle of the element serves as an introduction. More specific topics are examined in subsequent chapters on the presence of lead in atmospheric, terrestrial and aqueous environments. The geographical and geophysical applications of radioactive lead are the subject of a further chapter.

Of greater interest to the toxicologist is the second volume, which deals with the biological effects of the element. Here too a comprehensive approach is made and although the human health effects of lead take a lion's share, the toxicity of lead to domestic animals

and wildlife, aquatic biota, terrestrial microbiota and vegetation are also covered.

In man, apart from occasional episodes of lead poisoning, particularly in young children, acute exposure is a diminishing problem. The greatest concern at present is the possibility that continuous exposure to low levels may result in adverse health effects. Does subtle CNS impairment occur before effects are observed in the haemopoietic system? Questions such as this are examined in chapters on the human health effects of lead and on the metabolism and subclinical effects of lead in children.

One inevitable criticism can be aimed at this collation of information. As with any other intensively researched subject, the information is soon out of date. Nevertheless, the publication should prove to be extremely useful, and the editor does point out that the exercise has produced a sketch rather than a true portrait. We can look forward to the other proposed publications in this series.

Neurotoxicology. Vol. 1. Edited by L. Roizin, H. Shiraki and N. Grrcčević. Raven Press, New York, 1977. pp. xxviii + 658. $66.00.

The editors of this volume have presented a series of review articles on a wide range of chemicals specifically associated with adverse effects on the central and peripheral nervous system. The compounds selected for discussion represent important groups of chemicals. Many are pharmacologically active, exerting a tranquillizing, narcotic, anaesthetic, stimulant, antidepressant or hallucinogenic effect on the central nervous system. Others are important industrial chemicals, and some of these, including some pesticides, lead compounds and organomercurials, are also major environmental contaminants. A third important group comprises the antimicrobial agents, including such important substances as chloroquine, nitrofurantoin, clioquinol and hexachlorophene.

An honest attempt has been made to make the coverage as complete as possible. Each chapter is liberally supplied with references, and there are numerous diagrams and electronmicrographs. Attention has been paid primarily to neurotoxic effects in man, but animal work receives serious consideration as well. It is interesting to note that in many cases the neuropathological manifestations in animals differ significantly from those of human disease. This situation differs from that in other organs, such as the liver and kidney, in which the changes found in man often resemble very closely those encountered in animals. Despite these differences, animal models of chemical neurotoxicity have contributed to the understanding of neurotoxicity in man, and several examples of such contributions can be found in this book.

The introduction, by Leon Roizin, is well worth reading. It contains a brief historical sketch of the post-war growth of drug use and abuse, relating this and the technological advance of the chemical industry to the growing need for more understanding of the adverse actions of drugs on the central nervous system in man. The difficulties of acquiring such understanding are formidable, and Dr. Roizin has attempted to place in perspective the reasons for using animals in toxicological and pharmacological research and the value of such studies both in promoting the understanding of adverse drug reactions and in assessing hazard. In particular he stresses the value of facts established by valid experimentation of this sort, setting the scene with a quotation from Rudolph Virchow: "The recognition of the authority of the fact, the justification of the particular and the rule of the law".

Occupational Health and Safety Concepts. Chemical and Processing Hazards. G. R. C. Atherley. Applied Science Publishers Ltd., London, 1978. pp. xii + 408. £25.00.

Although expertise in toxicology is becoming a very saleable commodity, there is still a need for good introductory texts in many aspects of the science. The present volume, based on an Aston University course on Occupational Health and Safety, should fill at least one of the gaps in the bookshelf of the toxicological novice with no formal biological or medical training.

Essentially the book consists of two parts, the one concerned with science skates over the pertinent areas of physiology, biochemistry, immunology and pathology, whilst the other introduces the legislative and social aspects of occupational health through a series of case histories. The text is supported by a large number of well-chosen figures and illustrations.

Professor Atherley has attempted, largely successfully, to provide a basic foundation in industrial toxicology that will facilitate and encourage further study. To provide even a grounding in such a diverse subject in about 400 pages is obviously a difficult task, but it is only on rare occasions that the need for brevity leads to an unsatisfactory end product.

The major reservation about the book concerns its price; £25 is a lot to ask for a student text. Other specific criticisms are minor—an irregular and sometimes confusing subdivision of chapters and the introduction of an unnecessary and largely unhelpful "input-output model" in a discussion of a number of general points certainly do not outweigh the book's general virtues. In summary, then, this is a text to be recommended for the thrifty individual embarking on a study of industrial hygiene.

Report of the Government Chemist 1977. Department of Industry: Laboratory of The Government Chemist. HMSO, London, 1978. pp. iv + 173. £3.00.

Investigations into possible consumer hazards formed an interesting part of the Laboratory's work during 1977. As chromate is frequently used to prime non-stick cooking ware before it is coated with PTFE, a study of the chromium content of water boiled for an hour in such utensils was undertaken. In some cases hexavalent chromium was found at levels up to 1 mg/litre, but DHSS toxicologists advised that this would not present a hazard. The leaching of lead and cadmium from ceramic ware was also examined, but all results were well within proposed EEC limits.

Analysis for toxic metals in paint films on imported pencils and paintbrushes similarly gave little cause for concern, except for three samples which exceeded the limit for barium prescribed in the Pencils and Graphic Instruments (Safety) Regulations 1974.

Tests for leachable constituents in the British Standard specification for babies' dummies (BS 5239: 1975) were found to be inadequate for statutory control, and their revision is therefore to be undertaken. Of six dyestuffs used in a box of imported felt-tipped pens, two were considered undesirable if they might be licked by young children, and a wider survey of other constituents is planned. More unusual items examined during the year were a novelty lamp containing organic liquids, and a moulding kit which gave off styrene on heating.

The Laboratory has for a long time been engaged in the study of pesticide residues in the total diet, and the method of survey has been modified recently to take into account changes in eating habits. Since 1966 the mean daily intake of DDT and its metabolites in the total diet has fallen considerably, and in the period 1975–77 it was down to 5 μg (only 1/70 of the ADI for a 70-kg person). Levels of γ-BHC (lindane) and malathion have also fallen, and again were only a small fraction of the ADI. Hexachlorobenzene (recently measured for the first time) also gave no cause for concern, with a mean daily intake below 1 μg. Only in the case of dieldrin have intakes not declined since 1970; at 2·3 μg these were of the same order as the ADI (7 μg). The continued persistence of dieldrin in foods of animal origin, despite restrictions on its agricultural use since 1974, raises the question of a possible origin in other uses.

As the erucic acid content of edible oils and fats has been limited by recent legislation, a survey of 47 commercial margarines and cooking oils was undertaken. More than 5% erucic acid was detected in only four samples.

The Laboratory has also been assessing the suitability of new lacquer systems for canning fruit and vegetables. It has been concluded that electrolytic plate coated with an oleoresinous/epoxyphenolic lacquer is suitable for canning green beans but not for other acidic foods, and that acrylic lacquer, although improving protection against tinplate attack, leads to unacceptable deterioration of the product.

In the Toxic Substances in Air Sub-division, revision of the tests for benzene and isophorene has continued. In the former case an entirely new test has been deemed necessary. Work has also started on assessing the suitability of commercially available instruments for use as personal continuous monitors for aromatic isocyanates.

Aflatoxins: Chemical and Biological Aspects. By J. G. Heathcote and J. R. Hibbert. Elsevier Scientific Publishing Company, Amsterdam, 1978. pp. ix + 212. Dfl. 120.00.

In 1960, fungal metabolites responsible for mass outbreaks of poisoning of poultry in the south-east of England were isolated and called 'aflatoxins'. The carcinogenicity of aflatoxins is now well known and it is widely recognized that toxic products from moulds constitute a serious environmental hazard that cannot entirely be eliminated.

This book collates different aspects of the chemistry and biology of the aflatoxins. The authors deal with the biosynthesis and laboratory production of these mycotoxins, with their chemistry and with techniques for aflatoxin assay. The pathological effects of the aflatoxins and the particular mechanisms by which they damage susceptible cells are discussed and it is concluded that aflatoxins will be implicated in more cases of human cancer.

In a final section, the authors look at the control of aflatoxins and discuss methods available for their removal or destruction. They stress that as prevention is better than cure, detoxification should be considered a last line of defence and cannot be regarded as an alternative to good agricultural practice.

The presentation of this book is lucid and the reader should have little difficulty in following the account for the discovery of the aflatoxins through to the methods for controlling and preventing the growth of the fungi.

Alternatives to Animal Experiments. By D. H. Smyth. Scolar Press, London, 1978. pp. 218. £2.90.

Professor Smyth's book gives a general coverage of the alternatives to experiments with animals in all aspects of biomedical research. The presentation is aimed mainly at the layman, the issues are described clearly and the book is well-written. Simple explanations of the alternative techniques are given and elementary chapters are included on "living matter" and "biomedical research".

Although the subject is highly emotive, this book has been written with considerable objectivity. A biographical note is added so that the reader can assess for himself the likely extent and direction of the bias of the author in view of his lifelong involvement in biomedical science. The views of many involved individuals, who were consulted by Professor Smyth, are represented, and reflected in his conclusions. The report was commissioned by the Research Defence Society but was neither approved nor censored by that group.

The 'alternatives' discussed include dummies, models and computers, *in vitro* methods, tissue culture, lower organisms, man, the use of fewer animals, mass spectrometry and gas chromatography, audiovisual aids, and saturation analysis and radioimmunoassay. Alternatives are also considered specifically in relation to the cosmetic industry, the behavioural sciences, bacteriology, urology and immunology, and teratogenicity testing. The point that arises repeatedly in these discussions is that in many cases alternatives merely provide another approach to the problems being tackled or allow more information to be obtained from animal experiments; in most cases no 'alternative' can provide the information derived from whole animals.

No attempt is made to discuss the moral or ethical problems involved. Professor Smyth points out that the moral or ethical decision about whether the price of obtaining knowledge is too high is one for the individual. The decision about whether the knowledge

can be obtained in some other way is the scientific one and this requires careful examination of all the evidence. He also concludes that no alternatives to animal experiments for toxicity are compatible with the present safety standards demanded by Parliament, and clearly feels that to concentrate on finding alternatives to experiments that cause pain or distress to animals has the greatest chance of success. The final chapter of the book lists the conclusions and also includes a valuable list of suggestions to all interested bodies.

Drug Metabolism—From Microbe to Man. A Symposium in Honour of Richard Tecwyn Williams. Edited by D. V. Parke and R. L. Smith. Taylor and Francis Ltd., London, 1977. pp. xii + 460. £25.00.

This book consists of a series of 24 research papers presented at a symposium held at the University of Surrey, Guildford, during April 1976. The symposium was held in honour of Professor R. T. Williams, who recently retired from the Chair of Biochemistry at St. Mary's Hospital Medical School, London. Much of our present knowledge on the metabolism of xenobiotics stems from the pioneering work of Professor Williams and his colleagues, including his publication in 1947 of the first 'classic' textbook on the subject entitled *Detoxication Mechanisms—The Metabolism of Drugs and Allied Organic Compounds.*

The enzymic processes by which xenobiotics are metabolized in the mammalian liver were broadly divided by Professor Williams into two categories. Phase I biotransformations, involving enzymes of the microsomal cytochrome *P*-450-dependent mixed-function oxidase complex, include oxidative, reductive and hydrolytic reactions; the resultant metabolites may be conjugated with various endogenous acceptors by Phase II enzymes.

Aspects of both Phase I and Phase II pathways of xenobiotic metabolism were covered by papers presented at the Guildford symposium. For example, the paper on carbon oxidation by D. M. Jerina and J. W. Daly deals with epoxides as intermediates in the aromatic hydroxylation of xenobiotics and with the importance of such arene oxides in the activation of polycyclic hydrocarbons to carcinogens. A paper by A. H. Beckett considers various aspects of the oxidation of nitrogen in foreign compounds, and the factors responsible for the control of xenobiotic-metabolizing enzymes are considered by D. V. Parke, Papers by G. J. Dutton and co-workers, by K. S. Dodgson and by P. L. Grover deal with the three important Phase II pathways of conjugation with D-glucuronic acid, sulphate and glutathione, respectively.

As the title suggests, this volume aims to cover xenobiotic metabolism in all species, and various papers are devoted to metabolism by man, non-human primates, birds, fish, invertebrates, plants and micro-organisms. In addition, aspects of species differences in both Phase I and Phase II reactions are considered in a number of the papers. Pharmacokinetics, pharmacogenetics and the use of cell suspensions and cultures for metabolic studies are also discussed.

In general, the papers are essentially review articles considering the basic principles of various aspects of the subject, rather than presentations of the authors' latest research findings. In this respect this volume differs from the series entitled *Microsomes and Drug Oxidations* reviewed earlier (*Cited in F.C.T.* 1970, **8,** 546; *ibid* 1975, **13,** 270; *ibid* 1978, **16,** 383). Furthermore, the extraction of information from the book under review is greatly assisted by the excellent author *and* subject indexes. A publication to be recommended.

Pathology of Laboratory Animals. Vols I & II. Edited by K. Benirschke, F. M. Garner and T. C. Jones. Springer-Verlag, Berlin, 1978. pp. 2225. DM 565.30 (2 vols).

The need for a good textbook on the pathology of laboratory animals was felt several decades ago when experimental pathology and biochemistry were in their infancy. R. Jaffé gallantly responded to this need and in 1931 gathered the information then available into the first textbook of laboratory animal pathology. This remained the only reference book on this topic until 1958 when, together with P. Cohrs and H. Meessen and helped by a large group of collaborators, he produced a much enlarged version of his earlier work. Both of these excellent texts were written in German.

Since 1958, some major advances have taken place in pathology and, furthermore, an increasing burden has been imposed on the pathologist by demands from consumers, industry and government agencies for safety assurances. These developments have made the availability of an up-to-date text, preferably written in English, an urgent necessity. An attempt to tackle part of the problem was made some years ago by E. Cotchin and F. J. C. Roe, who, in collecting together a series of articles on rodent pathology, provided considerable help for the harassed pathologist, but they left some important gaps unfilled.

The two volumes named above go a long way towards meeting this urgent need. The first, which may be identified as dealing with 'systematic pathology', considers the disease processes in the so-called systems common to all mammals (the cardiovascular, nervous and respiratory systems, for example). The material presented is concerned not only with commonly occurring pathological features but also with more esoteric pathology. The latter is particularly important in toxicology, since rare lesions are more likely to be attributed in error to a test chemical than are commonly occurring ones.

The second volume of this pair is devoted to special topics. The infective diseases are the most important among these, and a succession of contributors deals succinctly with the various disease processes caused by lower organisms, from viruses to protozoa and helminths. Somewhat surprisingly, tumours are included in this volume. Neoplasia is a proliferative disorder, and one of the major problems in pathology is to distinguish it from non-neoplastic proliferative conditions. It would therefore be an advantage if, in future editions, the chapter on tumours could be included with the systematic pathology where conditions associated with non-neoplastic proliferation are

considered. On the other hand, it is recognized that these books do not form two related volumes in a series, but a single work bound in two separate parts of roughly equal and convenient size, and from this viewpoint the transfer of one, very long, contribution could present practical difficulties.

Considerable emphasis is placed on congenital abnormalities, in Volume II, and on disease processes resulting from chromosomal abnormalities (mutagenesis). These important topics are now universally considered to be growth areas in pathology, and the information contained in these sections will be invaluable to those interested in teratology and genetic toxicology.

Other chapters that are particularly worthy of note are those on clinical biochemistry, nutritive and metabolic disorders, and immunopathology. These are very broad topics in their own right, and the chapters are not long enough to give adequate coverage, but they serve at least to introduce the beginner to these important areas and to indicate the place they occupy in modern-day pathology.

These volumes are very well produced, with a good supply of clear photographs, which include a number of electronmicrographs, particularly in the section dealing with virus diseases. The pictures, especially those presenting histopathology, are arranged in groups depicting in each case the salient features of a pathological process. The addition of a good legend makes this arrangement an extremely useful adjunct to the description in the text.

This publication is not one that can be ignored by scientists engaged in experimental work on animals. Although its greatest value will be to the diagnostic pathologist, other scientists will find in it a wealth of information, which may help to tide them over many a pathological crisis!

BOOKS RECEIVED FOR REVIEW

IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Vol. 18. Polychlorinated Biphenyls and Polybrominated Biphenyls. International Agency for Research on Cancer, Lyon, 1978. pp. 140. Sw.fr. 20.00 (available in the UK through HMSO).

Toxicity of Heavy Metals in the Environment. Part 1. Edited by F. W. Oehme. Marcel Dekker, Inc., New York, 1978. pp. x + 515. Sw.fr. 106.00.

Continuous Culture 6: Applications and New Fields. Edited by A. C. R. Dean, D. C. Ellwood, C. G. T. Evans and J. Melling. Ellis Horwood Ltd., Chichester, 1976. pp. xi + 364. £15.50.

Water Pollution Microbiology. Vol. 2. Edited by R. Mitchell. John Wiley & Sons, Chichester, 1978. pp. x + 442. £17.60.

Moulds, Toxins and Food. By C. Moreau. Translated and edited by M. Moss. John Wiley & Sons, Chichester, 1979. pp. xiii + 477. £21.00.

Editing Scientific Books and Journals. An ELSE-Ciba Foundation Guide for Editors. By M. O'Connor. Pitman Medical Publishing Co. Ltd., Tunbridge Wells, 1978. pp. vi + 218. £7.00.

World Review of Nutrition and Dietetics. Vol. 29. Toxicology and Nutrition. Edited by R. Truhaut and R. Ferrando. S. Karger AG, Basel, 1978. pp. x + 190. DM 119.

Suspected Carcinogens. A Sourcebook of the Toxic Effects of Chemical Substances. Edited by E. J. Fairchild. Castle House Publications Ltd., Tunbridge Wells, 1978. pp. xxxi + 253. £20.00.

Nasopharyngeal Carcinoma: Etiology and Control. Edited by G. de-Thé and Y. Ito. IARC Scientific Publications No. 20. International Agency for Research on Cancer, Lyon, 1978. pp. xvii + 610. Sw.fr. 100.00.

The Handbook of Cancer Immunology. Vol. 2. Cellular Escape from Immune Destruction. Edited by H. Waters. Garland STPM Press, New York, 1978. pp. viii + 276. $37.50.

Toxicology Biochemistry and Pathology of Mycotoxins. Edited by K. Uraguchi and M. Yamazaki. John Wiley & Sons, London, 1978. pp. viii + 288. £19.50.

Chemical Toxicology of Food. Developments in Toxicology and Environmental Science Vol. 3. By C. L. Galli, R. Paoletti and G. Vettorazzi. Elsevier/North-Holland Biomedical Press, Amsterdam, 1978. pp. viii + 387. Dfl. 108.00.

Directory of On-going Research in Cancer Epidemiology 1978. IARC Scientific Publications No. 26. Edited by C. S. Muir and G. Wagner. IARC, Lyon, 1978. pp. xiii + 550. Sw.fr. 30.00 (available in the UK through HMSO).

Toxic Organic Chemicals. Destruction and Waste Treatment. By E. E. Hackman III. Noyes Data Corporation, Park Ridge, NJ, 1978. pp. xiv + 317. $42.00.

A History of Microtechnique. The Evolution of the Microtome and the Development of Tissue Preparation. By B. Bracegirdle. Heinemann, London, 1978. pp. xiv + 359. £22.50.

Environmental Health Criteria 6. Principles and Methods for Evaluating the Toxicity of Chemicals. Part I. Published under the joint sponsorship of the United Nations Environment Programme and the World Health Organization. WHO, Geneva, 1978. pp. 272. Sw.fr. 28.00 (available in the UK through HMSO).

Dictionary of Microbiology. By P. Singleton and D. Sainsbury. John Wiley & Sons Ltd., Chichester, 1978. pp. 481. £17.50.

The Handbook of Cancer Immunology. Vol. 3. Immune Status in Cancer Treatment and Prognosis—Part A. Edited by H. Waters. Garland STPM Press, New York, 1978. pp. 434. $37.50.

The Handbook of Cancer Immunology. Vol. 4. Immune Status in Cancer Treatment and Prognosis—Part B. Edited by H. Waters. Garland STPM Press, New York, 1978. pp. 335. $37.50.

Residue Reviews: Residues of Pesticides and Other Contaminants in the Total Environment. Vol. 69. Edited by F. A. Gunther. Springer-Verlag, New York, 1978. pp. viii + 146. $22.00.

Fd Cosmet. Toxicol. Vol. 17, pp. 403 to 412
Pergamon Press Ltd 1979. Printed in Great Britain

Information Section

ARTICLES OF GENERAL INTEREST

VINYL CHLORIDE—PART 1: METABOLISM

The period of phrenetic activity—or at least sustained debate—on the status of vinyl chloride (VC) seems to have passed. Since technological feasibility eventually formed the basis of the first generation of VC legislation, the authorities are now biding their time, pending the next generation of biological data, which will allow an assessment of the hazards associated with the currently permitted levels. We last reviewed VC early in 1976 (Potter, *Fd Cosmet. Toxicol.* 1976, **14**, 347 & 498). In the intervening period the biochemists and microbiologists have demonstrated particular interest in this monomer.

Dose-related metabolism?

A preliminary study by Dow Chemical USA on the fate of unlabelled VC in the rat indicated that its metabolism was dependent on the level of exposure (Hefner *et al. Ann. N.Y. Acad. Sci.* 1975, **246**, 135). In the range 50–105 ppm the half-life was 86 minutes, whereas in the range 220–1167 ppm the half-life was 261 minutes. Following up this work, the same group investigated the metabolism of ^{14}C-labelled VC in the rat (Watanabe *et al. Toxic. appl. Pharmac.* 1976, **37**, 49). Rats exposed to 10 ppm VC for 6 hours eliminated 68% of the absorbed radioactivity in their urine and 2% (as VC) in the expired air over 72 hours; 6-hour exposures to 1000 ppm resulted in 56% of the dose appearing in the urine with 12% in the expired air as VC. The pulmonary excretion of the VC followed first-order kinetics with similar half-lives at both treatment levels: 20·4 minutes at 10 ppm and 22·4 minutes at 1000 ppm. Elimination of ^{14}C in the urine was more complex, being described by a two-exponential equation, but the rates of the initial phase were comparable; at 10 ppm the half-life was 4·6 hours while at 1000 ppm it was 4·1 hours. After 72 hours, 14–15% of the administered radioactivity of both the high and low doses was recovered from the carcasses, indicating, when correction is made for the amount of VC metabolized, that there is increased storage of radioactivity at higher doses.

The metabolism of VC administered by other routes appears also be to dose-dependent. Indeed an even greater disparity between the metabolism of high and low doses seems to follow oral administration. In another study by Watanabe *et al.* (*ibid* 1976, **36**, 339) from 59 to 68% of an oral dose of 0·05 or 1 mg VC/kg was converted to non-volatile urinary metabolites, with 9–13% appearing as expired CO_2 and only 1–2% being excreted by the lungs as unchanged VC. By contrast, 11% of the highest dose of 100 mg/kg was excreted in the urine, and 67% was excreted as VC (and 3% as CO_2) in the expired air. The pulmonary excretion at 100 mg/kg was biphasic, with half-lives of 14·4 and 40·8 minutes whereas at 0·05 and 1 mg/kg it was monophasic with a half-life of about 55 minutes.

Similar findings were reported by the ICI workers, Green & Hathway (*Chemico-Biol. Interactions* 1975, **11**, 545), low intragastric doses of VC being eliminated principally in the urine whilst higher doses were mainly excreted unchanged via the lungs. About three-quarters of an intubated dose of 250 μg [^{14}C]VC/kg was converted to non-volatile urinary metabolites and 12–15% was excreted in the expired air as CO_2 together with a few per cent as unchanged monomer. By contrast, at a dose of 450 mg/kg pulmonary excretion predominated, with 90% of the radioactivity being associated with unchanged VC and less than 1% with CO_2. The pulmonary elimination of monomer, shown to be proportional to the log of reciprocal dose, was completed within 5 hours, whereas both the excretion of CO_2 via the lungs and the elimination of non-volatile metabolites in the urine continued over 72 hours. Similar excretion kinetics were observed in rats that had been pretreated for 60 days with unlabelled VC at doses up to 30 mg/kg/day (Green & Hathway, *loc. cit.*).

Withey (*J. Toxicol. envir. Hlth* 1976, **1**, 381) was unconvinced that intubation was an acceptable method of administering VC in a metabolic investigation, for he observed wide inter-animal variability in his studies in which rats with a cannulated jugular vein were intubated with aqueous and oil solutions of VC. The uptake of VC was rapid, peak concentrations being achieved within 10 minutes with blood levels varying from 6 to over 40 μg VC/ml.

The metabolism of VC given by iv and ip injection was also studied at ICI (Green & Hathway, *loc. cit.*). Almost all of a small (250 μg/kg) iv dose was found in the expired air as VC. About 43% of the same dose given by ip injection was excreted in the expired air as VC, another 43% being converted to urinary metabolites and 11% to CO_2. An ip dose of 450 mg VC/kg was almost totally excreted (96%) as VC in the expired air, with only 2·6% being metabolized to non-volatile species excreted in the urine.

Identity of metabolites

Identification of the urinary metabolites was attempted in both the Dow and ICI studies. In the oral and inhalation studies of Watanabe *et al.* (*loc. cit.*), most of the radioactivity excreted in the urine of rats was associated with three compounds, the proportions of which were unaffected by the dose or by the route by which the VC was administered. *N*-Acetyl-*S*-(2-hydroxyethyl)cysteine accounted for 29–40% and thiodiglycollic acid for 18–26% of the radioactivity detected in the urine, with the unidentified third compound being present at levels of from 30 to 39%. In the oral experiments, the three metabolites repre-

sented from 91 to 95% of the total radioactivity excreted in the urine, whilst in the inhalation experiments the corresponding figure was 96–97%.

The first report by Green & Hathway (*loc. cit.*) of their investigations of the metabolism of orally administered [^{14}C]VC (150 mg/kg) in the rat, noted that the three major urinary metabolites were thiodiglycollic acid (47% of the urinary radioactivity), *S*-(2-chloroethyl)cysteine (23%) and *N*-acetyl-*S*-(2-chloroethyl)-cysteine (23%). The remaining radioactivity in the urine was associated with urea (6%), glutamic acid (0·5%), chloroacetic acid (0·5%), methionine (trace) and serine (trace). Watanabe *et al.* (*Toxic. appl. Pharmac.* 1976, **36**, 339) were critical of these results, suggesting that the chloroethyl compounds were artefacts formed by the reaction of the hydroxy analogues with the hydrochloric acid gas used in the derivatization procedure. This criticism was accepted by Green & Hathway who reported in their most recent paper (*Chemico-Biol. Interactions* 1977, **17**, 137) that the major urinary metabolites of VC in the rat were *N*-acetyl-*S*-(2-hydroxyethyl)cysteine, the related *N*-acetyl-*S*-vinylcysteine and thiodiglycollic acid.

Further elucidation of VC's metabolic pathway(s) was attempted by Green & Hathway (1977, *loc. cit.*), who measured the yields of thiodiglycollic acid in the urine of rats given the various possible metabolites intragastrically at high doses. Thiodiglycollic acid in 0·5% theoretical yield was isolated in the urine of rats dosed with *S*-(2-hydroxyethyl)cysteine, a result said to be highly significant in view of the cysteine derivative's instability in even mild reaction conditions. Furthermore, as high yields of the acid were found in those animals dosed separately with chloroacetaldehyde, chloroacetic acid and *S*-(carboxymethyl)cysteine, it was suggested that all these compounds might lie on a common metabolic pathway linking VC with thiodiglycollic acid. Nevertheless, the fact that only small amounts of chloroacetic acid (<0·1%) were detected in the body fluids of animals treated with the high doses of VC, backed by evidence from the metabolism of chloroacetaldehyde (and vinylidene chloride), suggested that chloroacetic acid is not a major VC metabolite. Green & Hathway (1977, *loc. cit.*), believed that in rats, chloroethylene oxide was formed from VC and might then be transformed spontaneously into chloroacetaldehyde. The chloroacetaldehyde or chloroethylene oxide reacts principally with glutathione, catalysed by glutathione-*S*-epoxide transferase, to form *S*-(2-acetal)cysteine with subsequent formation of *S*-(2-hydroxyethyl)cysteine and its *N*-acetyl derivative, *S*-(carboxymethyl)cysteine and thiodiglycollic acid.

It was the view of Hefner and his colleagues (*loc. cit.*) in 1975 that VC was metabolized at low exposures, below 100 ppm, to 2-chloroethanol, chloroacetaldehyde and monochloroacetic acid via the alcohol dehydrogenase pathway. Only small amounts of the chloroacetic acid were formed at these low doses, due to the rapid reaction of chloroacetaldehyde with the sulphydryl groups of glutathione and cysteine. It was thought that at higher exposures (200–1000 ppm) direct epoxidation by microsomal oxidases occurred, with rearrangement of the resulting chloroethylene oxide to chloroacetaldehyde. However, the more recent study of Bolt *et al.* (*Arch. Tox.* 1976, **35**, 153) showed that in rats exposed to the monomer at an initial atmospheric concentration of 50 ppm in a closed system, the uptake of VC was completely blocked initially by 3-bromophenyl-4(5)-imidazole or 6-nitro-1,2,3-benzothiadiazole, both inhibitors of cytochrome *P*-450-dependent metabolism and was increased by DDT pretreatment, suggesting that the microsomal oxidases may be operative even at low doses. Weaker inhibitors of this enzyme system, such as SKF-525A or 5,6-dimethyl-1,2,3-benzothiadiazole, produced a measurable but lower order of inhibition. In the original Dow work (Hefner *et al. loc. cit.*), SKF-525A had no effect on the metabolism of low doses of VC (65 ppm) but did slightly decrease metabolism at VC exposures of the order of 1000 ppm.

Cytochrome P-*450 involvement*

Liver microsomal fractions have been shown to interact with VC *in vitro* (Salmon, *Cancer Lett.* 1976, **2**, 109). The addition of VC to rat-liver microsomes and NADPH produced a Type I spectral shift, similar to that seen for phenobarbital, thus indicating direct involvement of a cytochrome *P*-450 species. Other *in vitro* studies have also demonstrated that the oxidation of VC to non-volatile products was dependent both on microsomal enzymes and on NADPH (Kappus *et al. Toxic. appl. Pharmac.* 1976, **37**, 461).

It has been suggested that VC metabolites can destroy cytochrome *P*-450. Ivanetich *et al.* (*Biochem. biophys. Res. Commun.* 1977, **74**, 1411), for example, showed not only that the binding and metabolism of VC *in vitro* was fully inhibited by SKF-525A, indicating the involvement of the cytochrome *P*-450 system, but that the VC metabolites reduced the level of both cytochrome *P*-450 (reduced to *P*-420) and microsomal haem. The levels of cytochrome b_5 or NADPH-cytochrome *c* reductase were unaffected by exposure to the monomer. Decreases in the level of cytochrome *P*-450 and in the total activity of hepatic microsomal enzymes were observed in rats 24 hours after they had been exposed for 6 hours to a 5% VC atmosphere (Reynolds *et al. Res. Commun. chem. Path. Pharmac.* 1975, **12**, 685). The selective enzymatic deactivation was consistent with a cytochrome *P*-450-centred activation of VC to reactive electrophiles. The destruction of cytochrome *P*-450 was found to be inhibited by glutathione in the study of Ivanetich *et al.* (*loc. cit.*), whereas Guengerich & Strickland (*Molec. Pharmacol.* 1977, **13**, 993) reported that neither added reduced glutathione nor cysteine offered any protection against the VC-mediated destruction.

The loss of cytochrome *P*-450 activity was attributed by Guengerich & Strickland (*loc. cit.*) to the destruction of haem, and not to lipid peroxidation or the binding of electrophiles to free sulphydryl groups. Since the destruction of *P*-450 required all the components necessary for mixed-function oxidation, it was said that the oxidative metabolism of VC by cytochrome *P*-450 was necessary for the observed destruction. The investigators did not consider, however, that the two commonly proposed VC-metabolites, the epoxide and chloroacetaldehyde, were the agents responsible for the cytochrome *P*-450 loss, as neither compound proved particularly effective at destroying either free or *P*-450-bound haem *in vitro*. Consequently it was postulated that different

mechanisms underlie VC's mutagenic activity and its ability to destroy *P*-450. The latter is localized at the activating enzyme, consistent with a highly reactive species, whereas mutagenesis seems to require a metabolite stable enough to be transported from its site of activation to interact with nucleic acids or associated macromolecules.

Comment

The view that the microsomal mixed-function oxidases play an active and possibly the major role in the metabolism of VC monomer has received support from the demonstration that a microsomal-oxidase pathway operates at low VC exposures. The results of *in vitro* studies have also indicated that VC is metabolized by enzymes of the liver microsomes, in particular by a cytochrome *P*-450 species, to give initially chloroethylene oxide and 2-chloroacetaldehyde. Further investigation into VC-mediated destruction of cytochrome *P*-450 is required, but it would seem that here, too, a cytochrome enzyme is involved in the formation of the active metabolite(s).

The metabolic studies conducted by Dow and ICI have confirmed the dose-dependence of the fate of VC in the rat. It would seem, on the evidence now available, that in the rat there is effectively a single saturable metabolic pathway for VC. As the dose of VC increases, an increased proportion escapes metabolism and is eliminated unchanged in the expired air. The fact that the major urinary metabolites—probably derived from the conjugation of chloroethylene oxide or 2-chloroacetaldehyde with glutathione—appear to be unaffected both qualitatively and quantitatively by dose tends to oppose the hypothesis of alternative pathways varying in importance in relation to dose. If there are a number of important metabolic pathways, then they appear to be leading to the same end products, an unlikely, although not impossible, occurrence.

[J. Hopkins—BIBRA]

GETTING RID OF PENTACHLOROPHENOL

As a bactericide, fungicide and herbicide, pentachlorophenol (PCP) finds use in a wide range of applications; in particular, it has become an important wood preservative. Its potential significance as a major environmental contaminant must therefore be recognized.

PCP appears to be readily absorbed through the skin and has been held responsible for aplastic anaemia, muscular paralysis and sensory loss in factory workers who failed to use adequate protective clothing and who worked in poorly ventilated areas (*Cited in F.C.T.* 1965, **3**, 845). The toxicity of PCP to rats was shown to be greater when the compound was inhaled than after oral or subcutaneous administration (*ibid* 1977, **16**, 204). The question of PCP toxicity is complicated, however, by the fact that technical-grade PCP is frequently contaminated with varying amounts of toxic by-products, including other chlorinated phenols and, in some cases, chlorodibenzo-*p*-dioxins and chlorodibenzofurans. Thus Kimbrough & Linder (*Toxic. appl. Pharmac.* 1978, **46**, 151) found that whereas technical PCP fed for 8 months at a level of 500 or 100 ppm in the diet caused pronounced morphological changes in the livers of rats and some change was noted even with 20 ppm, no change was detected in rats fed 20 or 100 ppm purified PCP and only slight changes were apparent in those given 500 ppm in the diet.

Nevertheless, PCP itself has been shown to be an extremely efficient uncoupler of oxidative phosphorylation in mitochondria (Weinbach, *J. biol. Chem.* 1954, **210**, 545; Weinbach & Garbus, *Nature, Lond.* 1969, **221**, 1016) and to be capable of disturbing microsomal detoxication mechanisms in the liver (Arrhenius *et al. Chemico-Biol. Interactions* 1977, **18**, 35). By gas-chromatographic analysis of the sub-cellular fractions of the livers of rats treated orally with 0·15 mmol (*c.* 40 mg) PCP/kg, the latter group demonstrated that, compared with the cytosol, the mitochondria contained markedly lower concentrations of PCP and the microsomes showed a high accumulation (*idem, ibid* 1977, **18**, 23), suggesting that the effects of PCP on the detoxication functions of the endoplasmic reticulum are at least as relevant physiologically as the compound's capacity for mitochondrial uncoupling.

The fate of a single oral dose (10 or 100 mg/kg) of ^{14}C-labelled pure PCP was studied in rats for 9 days, with a final examination of tissues for residual activity (Braun *et al. Toxic. appl. Pharmac.* 1977, **41**, 395). The primary route of excretion of PCP was through the kidneys, with most of the remaining fraction of the dose appearing in the faeces. Only 0·2% of the lower dose appeared as expired $^{14}CO_2$. Male rats excreted 80% of the lower dose and 72% of the higher dose in the urine, and some 19 and 24%, respectively, in the faeces, while females excreted 78 and 54%, respectively, in the urine, and 19 and 43%, respectively, in the faeces. In the first 24 hours after administration of the higher dose, the urine contained 48% of the dose as unchanged PCP and 10% as tetrachlorohydroquinone (TCHQ). In both sexes given 10 mg PCP/kg and in males given 100 mg/kg elimination was biphasic; more than 90% of the total excretion occurred during the rapid elimination phase, with a half-life of 17 and 13 hours in males on the lower and higher dose respectively, and 13 hours in females given 10 mg/kg. Elimination of the 100-mg/kg dose in females was monophasic, with a half-life of 27 hours.

The highest residual concentrations in tissues were in the liver and kidneys, the lowest in the brain, spleen and fatty tissue. Females consistently showed higher plasma concentrations of PCP than did males, and some 99% was bound to plasma proteins. While this binding apparently slowed the urinary excretion of PCP, PCP glucuronide and TCHQ were readily excreted and their concentrations in plasma were much lower than those in the urine. The rate-limiting step for their elimination therefore appears to be their

formation by PCP metabolism rather than their urinary excretion, and any accumulation of a PCP metabolite in the body is highly unlikely.

More recently, this group has reported on the fate of PCP in man (*idem, ibid* 1978, **45,** 278; paper presented at the 1978 Meeting of the Society of Toxicology). In four healthy male volunteers who each ingested 0·1 mg PCP/kg body weight, the half-lives for absorption and elimination of PCP from plasma were approximately 1 and 30 hours and those for PCP and PCP glucuronide elimination in the urine were 33 and 13 hours, respectively. Within 168 hours of ingestion, about 74 and 12% of the dose had been excreted in the urine as PCP and its glucuronide, respectively. In addition, some 4% of the dose appeared in these two forms in the faeces. Urinary excretion reached a peak 42 hours after ingestion, while the maximum plasma concentration (0·248 μg/ml) was recorded at 4 hours. These findings fit a one-component open-system model with first-order absorption, enterohepatic circulation and first-order elimination, a model much more like that established for the rat than that derived from a study in monkeys. It was calculated that in men ingesting 0·1 mg PCP/kg daily, PCP would reach 99% of steady state in 8·4 days with a maximum plasma concentration of 0·491 μg/ml. There was no evidence to suggest that the toxic effects of PCP would be cumulative, even with repeated daily low-level exposures.

The data derived from a very different situation—an attempted self-poisoning by ingestion of PCP—were built into a pharmacokinetic model by Young & Haley (*Clin. Toxicol.* 1978, **12,** 41), with the aim of predicting an appropriate procedure for reducing tissue levels of the compound. This study indicated that forced diuresis by administration of frusemide (furosemide) and mannitol would materially reduce the body burden of PCP. Without diuresis the blood and tissue concentrations showed little tendency to decrease for about 48 hours after PCP ingestion. Using first-order decay equations, the time for seven half-lives to occur (leaving 0·78% of the original dose in the body) was calculated as 33·7 days in the absence of diuresis, and 12·3 days if diuresis were continued over the whole of the treatment period; in fact, in the case cited, diuresis began about 70 hours after the poisoning attempt and continued for 50 hours reduced this seven-half-lives period to 30·6 days.

The effect of microsomal-enzyme induction and inhibition on PCP metabolism has been investigated by Ahlborg *et al.* (*Arch. Tox.* 1978, **40,** 45). Rats were pretreated with 75 mg phenobarbital/kg ip for three consecutive days and on day 4 were given an ip dose of 10 mg PCP/kg. Others were pretreated with SKF-525A in a dose of either 25 or 50 mg/kg ip every 6 hours or less frequently. Phenobarbital pretreatment increased the conversion of PCP to TCHQ (identified as the *p*-isomer, TC*p*HQ) both *in vivo* and in microsomal preparations derived from the livers of the pretreated rats. SKF-525A inhibited PCP metabolism *in vitro*, but enhanced it *in vivo* if given less frequently than 6-hourly. Dechlorination of PCP to TCHQ seems, therefore, to be mediated by liver microsomal enzymes which can be induced by phenobarbital. The biphasic effect of SKF-525A, which appeared to involve initial inhibition followed by a stimulatory phase, has not been fully explained.

Experiments with another powerful enzyme inducer, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), which has been identified as a contaminant of some samples of technical PCP, have shown that more than one microsomal-enzyme system may be involved in the detoxication of PCP (Ahlborg & Thunberg, *ibid* 1978, **40,** 55). Rats were given 10 mg PCP/kg ip 4 days after pretreatment with either 10 μg TCDD/kg by gavage, 20 mg 3-methylcholanthrene/kg ip or 75 mg phenobarbital/kg ip, the latter treatment being repeated on five successive days. Pretreatment with phenobarbital increased excretion of TC*p*HQ but not of a second urinary metabolite, trichloro-*p*-hydroquinone. TCDD and methylcholanthrene pretreatments increased the excretion of both hydroquinones compared with that in controls, and recovered overall a greater proportion of the dose, but total recovery was not affected by phenobarbital pretreatment. Microsomal preparations from pretreated rats showed that PCP metabolism *in vitro* was increased nearly threefold by phenobarbital but more than fivefold by TCDD. The inhibitory effect of SKF-525A on microsomal metabolism was more pronounced after phenobarbital pretreatment than after TCDD pretreatment.

[P. Cooper—BIBRA]

THE KIDNEY AND OCHRATOXIN A

Balkan (or endemic) nephropathy is a disease characterized clinically by renal failure seldom accompanied by salt retention or hypertension. It is often fatal within 2 years of its onset. The major lesion is a progressive destruction of the renal tubules, accompanied by interstitial and capsular fibrosis and followed by gross renal atrophy (*Lancet* 1977, **I,** 683). Up to about one in ten of the rural population is affected in the areas, lying around the Danube and its tributaries in adjacent parts of Yugoslavia, Bulgaria and Rumania, in which the disease is endemic. About one third of patients dying from Balkan nephropathy in Bulgaria and Yugoslavia have been reported also to have papillomas and/or carcinomas of the renal pelvis, ureter or bladder.

Sattler *et al.* (*ibid* 1977, **I,** 278) have reported that over 23% of the 140 apparently healthy individuals in the Bulgarian village of Gorno Pestane, where both Balkan nephropathy and papillomas of the urinary tract are common, had raised serum and/or urine concentrations of β_2-microglobulin, a component of the low-molecular-weight proteins that feature in the proteinuria associated with the early stages of Balkan nephropathy. An excess of serum β_2-microglobulin

was detected in five of six patients with tumours of the renal pelvis. The finding in one population of a high incidence of two diseases associated with high β_2-microglobulin levels, either in the serum or urine, suggests the possibility of a common aetiology.

The search for the cause of this disease has covered various local factors, but no trace element in local soil has been implicated as an aetiological agent, and the disease existed prior to the introduction of synthetic agrochemicals. Neither is there any evidence of bacterial involvement. Suspicion has fallen, however, on certain nephrotoxic fungi that contaminate human foodstuffs (*Lancet* 1977, **I**, 683). *Penicillium verrucosum* var. *cyclopium* and certain Fusarium species have been identified in foods showing heavy fungal contamination, but the wide distribution of these fungi does not correlate with the restricted occurrence of Balkan nephropathy. On the other hand, the effects of ochratoxin A (OTA), a secondary metabolite of some Aspergillus and Penicillium species found mainly on cereals, have been demonstrated in animals, notably pigs and rats, and closely resemble those seen in Balkan nephropathy (*cited in F.C.T.* 1977, **15**, 246).

Krogh *et al.* (*Acta path. microbiol. scand. B* 1977, **85**, 238) sampled foodstuffs from an area in Yugoslavia where Balkan nephropathy is endemic, and from a control area where it is not. Samples containing OTA were found in both, but the OTA contamination of cereals was higher in the endemic area. Concentrations of 5–90 μg OTA/kg were found in grain. Moreover, one of 12 samples of pork from the endemic area contained 5 μg OTA/kg. This contamination was probably derived from animal feed, demonstrating how OTA may reach the human diet indirectly.

In animal studies on OTA derived from an *Aspergillus ochraceus* culture, Kanisawa *et al.* (*Toxic. appl. Pharmac.* 1977, **42**, 55) established an acute oral LD_{50} in rats of 28 mg/kg and demonstrated that severe damage to the renal tubules followed three or four daily doses of 5 or 10 mg/kg. Massive degeneration, with necrosis and desquamation of the epithelium, occurred in the proximal tubules, the earliest and most severe damage being evident in the pars recta. Barnes *et al.* (*Lancet* 1977, **I**, 671) force-fed cultures of *P. verrucosum* var. *cyclopium* as a homogenate to rats for 5 days/week for 4 weeks. This treatment was associated with lesions of the lower portions of the proximal convoluted tubules, resembling the changes seen in patients with Balkan nephropathy. The strain producing the nephrotoxin was isolated from stored maize grain. Elling & Krogh (*ibid* 1977, **I**, 1213) pointed out, however, that no one had shown unequivocally that the site of kidney lesions in clinical Balkan nephropathy was in fact the lower part of the proximal convoluted tubules, as Barnes *et al.* (*loc. cit.*) implied.

When chickens were fed 0·3 or 1 mg OTA/kg feed for 341 days, certain aspects of renal function were impaired (Krogh *et al. Acta path. microbiol. scand.* A 1976, **84**, 215). Impairment of glomerular and tubular function was indicated by a dose-related decrease in inulin clearance, by 12 and 28% in the two treated groups. The tubular excretion rate of PAH (*p*-aminohippurate) was not reduced at low plasma concentrations, but was reduced by 33% at higher ones. Renal concentrating capacity fell by about 15% in both treated groups. Histological examination of the kidneys showed degeneration of the tubular epithelium and subsequent partial regeneration. The chickens contained residues of OTA in kidney, liver and muscle, to a maximum of 50 μg/kg in the kidney and 9 μg/kg in muscle after exposure to the higher dietary level for only 2 weeks. Residues were lower after the full 341-day treatment. Since the birds carrying these OTA residues had no macroscopic lesion that might have lead to the rejection of the meat by a health inspector, it seems that a human health hazard could possibly arise from the feeding of chickens on OTA-contaminated feedstuffs.

In porcine studies carried out by the same group (*idem, ibid* 1976, **84**, 429), pigs fed 1 mg OTA/kg feed for 3 months developed a nephropathy resembling naturally occurring porcine nephropathy. The syndrome involved impaired proximal tubular function, indicated by a decrease in the ratio of the maximal tubular excretion of PAH to the inulin clearance, a decrease in concentrating capacity and an increase in glycosuria. Histological examination showed degeneration and subsequent atrophy of the proximal tubules, and interstitial fibrosis. Significant residues of OTA were found in kidney, liver, fat and muscle but, as in the case of the chickens, there were no macroscopic lesions that would have led health inspectors to reject the meat.

A series of studies of the biochemical, histological and ultramicroscopic lesions produced by citrinin and OTA in beagle dogs helps to fill in much of the detail. Kitchen *et al.* (*Vet. Pathol.* 1977, **14**, 154) gave 0·1 or 0·2 mg OTA/kg by mouth in a capsule and/or 5 or 10 mg citrinin/kg ip in ethanol, to young dogs daily for 14 days. Dogs survived the lower dose of citrinin but emesis and tenesmus occurred immediately after each injection; the higher dose was fatal within 7–11 days. Neither dose of OTA provoked any toxic signs. When OTA was given in combination with citrinin, the effects resembled those of 10 mg citrinin/kg given alone, namely anorexia, emesis, retching, tenesmus, polydipsia, polyuria and prostration. Four of six dogs given 5 mg citrinin/kg plus 0·1 mg OTA/kg died in 13–14 days, while those given 10 mg/kg plus 0·2 mg OTA/kg died within 4–5 days. In intoxicated animals, urinary glutamic–oxalacetic transaminase and lactic dehydrogenase were increased; serum lactic dehydrogenase was raised in those receiving 10 mg citrinin/kg. The larger doses of citrinin given with or without OTA produced cellular and granular casts in the urine, ketonuria, proteinuria and glycosuria.

The gross lesions observed included focal peritonitis and intestinal intussusception in the dogs given citrinin (*idem, ibid* 1977, **14**, 261). The higher dose of citrinin caused degeneration and necrosis, with desquamation of the epithelial cells of the distal tubules and collecting ducts. Changes produced by OTA were similar, but were primarily in the straight segment of the proximal tubules. When citrinin and OTA were given together, the lesions appeared in both the proximal and distal tubules. OTA also produced necrotic changes in the lymphoid tissue of the spleen, tonsil, thymus, peripheral lymph nodes and ileal, colonic and rectal lymph nodes. The larger combined dose of

citrinin and OTA produced ulceration of the intestinal mucosa. It could not be established, however, whether the enhanced toxicity when both mycotoxins were given together was synergistic or merely additive.

In a third paper (*idem, ibid* 1977, **14,** 392), the ultrastructural changes in the kidneys of dogs given OTA or citrinin were compared. No changes were seen in the epithelial cells of the proximal or distal tubules of dogs given 5 mg citrinin/kg, but in those given 10 mg/kg the cells of the proximal tubules showed cytoplasmic vacuolation, myelin-figure formation and cytoplasmic disarray mainly affecting the endomembranous system, and cytoplasmic vacuolation together with some cytoplasmic disarray and mitotic figures was apparent in the cells of the distal tubules. In this group, some secondary mitochondrial swelling was seen in severely affected cells. The changes in the proximal epithelial cells after 0·1 or 0·2 mg OTA/kg were similar, but the cells of the distal tubular epithelium were not affected in these animals. Thus, the location of lesions within the nephron occurred primarily in the proximal tubules with OTA alone, and in the distal tubules with citrinin alone. When combined doses of both mycotoxins were given, lesions occurred in both parts of the tubule and the collecting ducts, but the citrinin-induced lesions predominated.

Balkan nephropathy, with its possible association with human diets contaminated with OTA, and Swedish porcine nephropathy, in which OTA appears to be a major aetiological factor, have been particularly associated with very specific geographical areas. Fungi capable of producing OTA under appropriate conditions are more widespread, however, and the arrival in Bournemouth of a consignment of flour contaminated with OTA was reported recently. Richardson *et al.* (*Lancet* 1978, **II,** 1366) found that some caked and discoloured culinary flour from two sacks that were the subject of a complaint contained 6·25 mg OTA/kg, as determined by thin-layer chromatography. It appeared that the flour had become damp during storage and this had caused proliferation of *P. cyclopium* and the consequent production of OTA. The OTA level quoted was limited to the obviously affected part of the flour, and it was estimated that the overall concentration in the sacks was below 1 mg/kg. This appears to be the first instance in which a high level of OTA has been detected in retail foodstuffs in the UK. The actual health risk in this case was apparently not serious and it should not be exaggerated. However, the incident does underline the need to maintain a careful watch for the possible occurrence of mycotoxins in foods and, as the authors of this report suggest, indicates the advisability of reconsidering the current practice of diverting into animal feeds milled flour that has become discoloured by mould contamination.

[P. Cooper—BIBRA]

AFLATOXINS AND THE LIVER

Fungi that synthesize aflatoxins, particularly *Aspergillus flavus* and *A. parasiticus*, are common natural contaminants of peanuts, cotton seed, soya beans, pecans, cereals, pulses, cassava and sweet potatoes. Among the different aflatoxins identified, aflatoxins B_1 and G_1 have produced liver cancer when fed to rats, while B_2 has produced liver tumours only when injected ip (*Cited in F.C.T.* 1976, **14,** 151). These mycotoxins have been shown to affect a wide range of animal species, and the susceptibility of primates has been demonstrated in several laboratory tests.

Following some early very small studies in which the feeding of mixed aflatoxins to rhesus monkeys was associated with the developement of hepatocellular or bile-duct carcinoma (Gopalan *et al. Fd Cosmet. Toxicol.* 1972, **10,** 519; Tilak, *ibid* 1975, **13,** 247). Adamson *et al.* (*J. natn. Cancer Inst.* 1976, **57,** 67) have reported that three of 42 rhesus monkeys developed primary liver cancers after receiving total doses of aflatoxin B_1 ranging from 99–842 mg over periods of 48–74 months, although no such cancers developed in 20 control animals and none had been detected in 250 untreated monkeys kept in the colony during the previous 15 years. An indication of the possible hazard of working with aflatoxin has come from Deger (*Ann. intern. Med.* 1976, **85,** 204), who reported that two men, aged 42 and 28 years, developed colonic carcinoma after spending 3 years and 1 year, respectively, scraping aflatoxin from chromatographic plates. Despite the impossibility of establishing a cause-and-effect relationship in these circumstances, the known carcinogenic potential of aflatoxins and the rarity of colonic adenocarcinoma in relatively young men combine to raise grave suspicion.

Investigations of the carcinogenic potency of aflatoxin in man, however, have been concerned mainly with the possible association between liver tumours and aflatoxin-contaminated diets. Epidemiological studies involving diet and tissue analyses have been conducted over the past decade in areas in which the incidence of primary liver cancer is particularly high. Some of the earliest work was carried out in Thailand and this was followed by several studies on various African populations (*Cited in F.C.T.* 1976, **14,** 152).

Following earlier work in Kenya, Peers *et al.* (*Int. J. Cancer* 1976, **17,** 167) studied exposure to aflatoxin among a Swaziland population whose main articles of diet were maize porridge, groundnuts, sour drinks and beers, supplemented with jugo beans, sorghum and various pulses. Samples of food were collected every 2 months for 1 year from the different areas selected to provide a range of climatic and socio-economic conditions and of food storage and handling methods. Analysis of these samples for aflatoxin and calculation of daily aflatoxin ingestion levels established a significant correlation between individual daily intakes of the mycotoxin and the incidence of primary liver cancer in adult men in different parts of Swaziland. Although the aflatoxin contamination of food is related mainly to poor storage, no seasonal

effect on the aflatoxin content of samples could be demonstrated, suggesting that the discriminatory sorting of maize and groundnuts and the consequent discarding of mouldy stocks reduces exposure to aflatoxin throughout the year to a fairly constant pattern. This report adds some detail to the pattern of the relationship of liver cancer to dietary aflatoxin intake already reported for other areas of Africa (*Cited in F.C.T.* 1976, **14,** 152).

Susceptibility to the acute toxicity and/or carcinogenic potential of aflatoxin varies widely between species and even among strains of the same species, and the relationship between these two aspects of aflatoxin activity remains far from clear (*ibid* 1978, **16,** 403). The signs of acute aflatoxicosis reported in the baboon, notably a varying degree of centrilobular necrosis, a diffuse inflammatory reaction and some proliferation of the bile-duct epithelium, were similar to those reported in other primates. They differed, however, from those seen in the rat and duckling, in which periportal necrosis is a prominent feature. An extensive outbreak of acute aflatoxicosis in man (apparently the only one so far recorded) occurred in India in 1975. Jaundice, oedema of the peritoneal cavity and lower limbs and severe gastro-intestinal bleeding caused a high death rate among a population subsequently discovered to have consumed maize contaminated with 6–15·6 μg/g aflatoxin (*ibid* 1976, **14,** 151).

Some 200 of the survivors of this outbreak of hepatitis were examined about a year later, monthly checks having been made in the intervening period by means of a reply-card system operated through local Medical Officers (Bhat & Krischnamachari, *Indian J. med. Res.* 1977, **66,** 55). Following the very high death rate during the acute phase of the illness, a further six deaths occurred during or after the subacute phase (between 6 and 8 months after the onset). Jaundice had persisted for 5–7 months in some cases. Among the 70 maize samples collected during the resurvey only seven contained aflatoxin, and then not in excess of 0·1 μg/g. Although these samples contained a number of fungi in addition to *A. flavus*, no other mycotoxins were definitely identified. A long-term follow-up designed to demonstrate any change in the incidence of cirrhosis or liver carcinoma in this area would be of considerable interest.

While aflatoxin has been demonstrated in human tissues originating from areas where food storage presents particular problems (Shank *et al. Fd Cosmet. Toxicol.* 1971, **9,** 501), it is unusual to find reports of aflatoxin in routine human biopsy specimens. However, Phillips *et al.* (*Toxic. appl. Pharmac.* 1976, **36,** 403) found aflatoxin B_1 in a concentration of 520 ng/g wet liver in a Missouri resident who suffered from carcinoma of the rectum and liver, with extensive hepatic necrosis. No other aflatoxins were detected in the biopsy specimen, but when a homogenate of the liver tissue was incubated with an NADPH-generating system, the aflatoxin B_1 recoverable by chloroform extraction was reduced to 250 ng/g wet liver and a highly polar metabolite appeared in a concentration of 40 ng/g. Stora (*C.r. hebd. Séanc. Acad. Sci., Paris* 1978, **286,** 917) has described a technique used for detecting aflatoxin in sections of tissue from primary liver tumours obtained from a population studied in Zaïre. The method, based on fluorescence microscopy, differentiates between the fluorescence of aflatoxins B_1, B_2 and their metabolites, and between that of aflatoxins G_1, G_2 and their metabolites.

The question of a possible association between Reye's syndrome and aflatoxin ingestion (*Cited in F.C.T.* 1976, **14,** 151) continues to evoke interest. A combination of acute encephalopathy with fatty degeneration of the viscera is particularly common in Thailand, where it affects hundreds of children. Many Thai foods contain aflatoxin, and the incidence of the disease corresponds with seasonal and geographical variations of dietary aflatoxin (Harwig *et al. Can. med. Ass. J.* 1975, **113,** 275). Autopsy specimens from 22 of a group of 23 Thai children with this syndrome showed the presence of aflatoxin B_1, the highest concentrations being in the liver (93 ng/g), stomach and intestinal contents (127 ng/g), stools (123 ng/g) and bile (8 μg/ml). Harwig *et al.* (*loc. cit.*) point out that oral administration of aflatoxin B_1 to macaque monkeys has induced a condition resembling Reye's syndrome both clinically and pathologically, while liver extracts from two children in New Zealand and two in Czechoslovakia who died of Reye's syndrome were shown to contain fluorescent compounds corresponding chromatographically with aflatoxins B_1 and G_1 and with aflatoxin B_1, respectively. Chaves-Carballo *et al.* (*Proc. Staff Meet. Mayo Clin.* 1976, **51,** 48) examined liver specimens from eight patients with Reye's syndrome, from two patients with acute encephalopathy without fatty liver, and from ten patients without evidence of liver disease. Thin-layer chromatography of liver extracts from 19 of these patients showed no fluorescence indicative of aflatoxins, but the extract from one patient showing the clinical, laboratory and pathological features of Reye's syndrome yielded a compound similar to but not identical with aflatoxin B_1. The liver concentration of this compound (calculated as aflatoxin B_1) was 22·5 ng/g. There was not enough of this compound for further characterization. Hogan *et al.* (*Lancet* 1978, **1,** 561) have reported that the blood of two children with Reye's syndrome contained aflatoxin B_1 during the acute phase of the disease. Both patients had suffered an initial viral illness and later developed vomiting, hyperventilation, enlarged liver, decerebrate posturing, seizures and coma. Their serum concentrations of the aflatoxin were 11·93 and 31·3 ng/ml respectively Both children died, and Reye's syndrome was confirmed in one *post mortem*; the other was not autopsied. The information obtained from these post-mortem studies, although limited, suggests that further analysis of tissue specimens from victims of Reye's syndrome would be a worthwhile field of study.

[P. Cooper–BIBRA]

HEXACHLOROPHENE: MORE BAD NEWS THAN GOOD

The whole-body bathing of the newborn with 3% solutions of hexachlorophene (HCP) became standard paediatric practice during the sixties. Throughout the previous decade outbreaks of serious staphylococcal disease were common in hospital nurseries and a marked reduction in staphylococcal infection coincided with the introduction of HCP prophylaxis. It was thus generally accepted that HCP bathing was a major reason for this improvement in neonatal health. A drastic curtailment in the routine use of HCP-containing soaps in nurseries following the 1972 pronouncements of the FDA provided an opportunity, perhaps the first opportunity, to assess objectively the efficacy and safety of this material as a bacteriostat.

Efficiency as a bacteriostat

There is still no general agreement as to the effectiveness of HCP against virulent staphylococcal disease in the newborn, although a number of papers have been published on the subject. An investigation by Hyams *et al.* (*Am. J. Dis. Child.* 1975, **129,** 595) indicated that staphylococcal colanization of the newborn in a crowded nursery of a US county hospital rapidly increased after regular HCP bathing was discontinued. Only 2·2% of the nasopharyngeal samples taken from a group of 92 infants who were bathed daily with 3% HCP gave evidence of staphylococcal colonization. There was a 46% incidence of *Staphylococcus aureus* in infants bathed with a 0·75% HCP solution; the corresponding incidence of colonization in a group washed daily with ordinary soap was 67%. Of the 43 types of Staphylococcus identified, 36 were classified as type 86 (but whether this strain is particularly virulent was not established). Some 10 weeks after this preliminary study had been completed, a period in which only soap bathing was used, eight babies who were present in or had been recently discharged from the nursery developed signs of staphylococcal disease. In an attempt to identify whether babies were suffering from occult disease when they were discharged, case histories were obtained from 523 of the 635 babies who had left the unit in the previous 2 months. Almost a third of those discharged were said to have been ill during this time. Of the 175 "ill" babies examined by medical staff, six showed purulence, mainly of the eye, and two others had a pustular skin rash. Cultures from each of these eight infants yielded *S. aureus*, seven isolates being type 86. In a further microbiological investigation, 11·8% of the 169 nasopharyngeal samples taken from the hospital staff were found to be positive for *S. aureus*; two of the isolates obtained from the nursing staff were type 86.

A similar outbreak of disease occurred in the nursery of a US University hospital, only 3 weeks after whole-body bathing of the newborn with HCP was discontinued (Najem *et al. ibid* 1975, **129,** 297). Sixteen of the 39 babies in the neonatal special care unit were affected, and 11 of these cases were shown to have been caused by staphylococcal infection. Phage types of ten strains of *S. aureus* were isolated from the lesions of nine of the infants suggesting that there was no single source of contamination. Two days after the last case of staphylococcal disease was reported, microbiological surveillance of the unit was initiated. During the first 3 weeks of surveillance, when HCP was not used but control measures consisting of reinforcement of basic nursery techniques were taken, an average of seven infants a week were found to be colonized with Staphylococcus. From week 4 to 6, when whole-body washing with 3% HCP took place every other day, three infants a week gave positive staphylococcal samples. During week 7, by which time daily HCP bathing had been reinstated, only a single infant became colonized with *S. aureus*.

Investigations by Gehlbach *et al.* (*Pediatrics, Springfield* 1975, **55,** 422) into an outbreak of streptococcal and staphylococcal skin disease at a US Medical Centre suggests that the relationship between this type of disease and HCP-usage may not be as straightforward as the results of Hyams *et al.* (*loc. cit.*) imply. Before December 1971 it was usual practice at the hospital to wash the infants in the full-term ward daily with 3% HCP. Two weeks after routine washing with HCP had been discontinued two babies who had been discharged from the unit developed omphalitis; cultures from both proved positive for Streptococcus. Eleven more cases of neonatal skin disease in infants born at the centre had occurred during the 4 months before HCP-bathing was stopped. Seven further cases of omphalitis and eleven of pustulosis were identified during the 2 months after HCP-bathing was stopped and were shown to be caused by either staphylococcal or streptococcal infection. A microbiological survey was then initiated and 95% of the babies discharged from the unit were found to be colonized with Streptococcus and/or Staphylococcus. While the Staphylococci were of many types, all the Streptococci examined were of a single type, indicating a single carrier, although attempts to identify a carrier were unsuccessful. Various control measures were then introduced in the nursery including reintroduction of HCP bathing in order to try and reduce bacterial colonization. Because high colonization rates recurred after only a short period of decline, it was decided that the HCP bathing was proving ineffective and it was discontinued. New control measures were then introduced directed to improving other hospital procedures and these resulted in an abrupt decline in bacterial colonization. Colonization rates of 10% were maintained until the end of the study.

A report by Light *et al.* (*J. infect. Dis.* 1975, **131,** 281) also suggests that in some circumstances there may be no direct association between the incidence of staphylococcal colonization and HCP prophylaxis. Before the advent of regular HCP bathing the incidence of colonization by Staphylococcus in the full-term nursery of a US General Hospital was 60–75%. During the period when HCP was used regularly, colonization decreased and was down to 6·6% by 1967. HCP then fell from favour and its use was discontinued; during the next 4 years the incidence of colonization gradually rose to 47%. However, colonization rates in the premature nursery of the same hospital showed a similar trend in spite of the fact that bathing of the newborn with HCP was never intro-

duced into this unit. During the period 1965–1967, colonization rates in the premature nursery decreased from 69 to 23%. In contrast to the full-term nursery this decline continued beyond 1967 and an 8·7% incidence of colonization was recorded in 1969. Similarly, the relative frequency of colonization of infants with *S. aureus* 80/81 (a type commonly associated with disease outbreaks) decreased during the period 1960–1972 in both full-term and premature infants.

Colonization of the newborn by the Staphylococcus strains that produce mild infections can be reduced by HCP prophylaxis on the evidence of the results of Hyams *et al.* (*loc. cit.*) and Najem *et al.* (*loc. cit.*). By contrast, the findings of Light *et al.* (*loc. cit.*) suggest that colonization by both the mild and virulent strains of the bacterium, strain 80/81 being capable of threatening life, do not appear to be significantly affected by HCP.

Staphylococcal disease is known to have an unpredictable epidemic periodicity. The results of Light *et al.* (*loc. cit.*) indicate that from 1965 to 1969 there was a natural decline in the virulence of the bacterium. Consequently the critical period when restrictions were first imposed on HCP may have coincided with a natural increase in the activity of *S. aureus*, with a corresponding increase in staphylococcal disease. It is also probable that the FDA action on HCP highlighted the problem of bacterial colonization of the newborn, and this in itself may have weighed the subsequent epidemiological data towards increased staphylococcal colonization and disease (Bressler *et al. Clin. Pediat.* 1977, **16**, 342).

Toxicity

While the benefits of HCP to the hospital nursery remain debatable, is there now some agreement on the risks involved in its use? Valuable information on the toxicity of HCP to the newborn was obtained by Plueckhahn & Collins (*Med. J. Aust.* 1976, **1**, 815) in a comprehensive study involving two Australian hospitals; the Royal Women's Hospital, where all newborn are bathed with HCP 12 hours after birth and every second day thereafter, and the Royal Children's Hospital where HCP is not used. From 1959 to 1969, 245 babies died at the Royal Women's Hospital after living for at least 2 days and at the Royal Children's Hospital there were 638 deaths in infants less than 4 months old. At the Royal Women's Hospital, 48 out of the 245 infants were found to have suffered from central nervous system (CNS) vacuolation; all of those affected had weighed less than 2 kg at birth and had been washed at least four times with 3% HCP. Of the 92 infants who died at the Royal Children's Hospital and who had a birth weight of less than 2 kg, 11 had vacuolation, and of these, nine had been washed with HCP in other hospitals while two had no history of HCP exposure. Vacuolation occurred in four infants who had a birth weight of over 2 kg but none had been bathed with HCP. Combining the data from both hospitals showed that 59 of the 188 premature babies weighing less than 2 kg at birth had vacuolation. Two of these had never been washed with HCP, nine of the 28 washed three times had vacuolation, and 48 of the 62 infants washed four or more times were affected. Of the 670 babies with a birth weight of over 2 kg, 146 had been washed three or four times with 3% HCP with no apparent damage to the CNS.

The data of Plueckhahn & Collins (*loc. cit.*) identify premature infants as the group at greatest risk from routine bathing with 3% HCP, babies with birth weights over 2 kg apparently being unaffected. Confirmation of the susceptibility of the premature baby to the neurotoxicity of HCP came from a study by Shuman *et al.* (*Archs Neurol., Chicago* 1975, **32**, 320), involving the blind clinico-pathological analysis of the CNS of 46 premature infants with birth weights of less than 1·4 kg who had died after surviving at least 4 days. This analysis suggested that repeated whole-body bathing with 3% HCP solution was associated with a significant increase in vascular encephalopathy of the brain stem. The prevalence of the lesion appeared to be related to the number of exposures to the bacteriostat, its concentration, and to the thoroughness of rinsing.

Evidence that vacuolation of the CNS may not simply be related to HCP exposure, however, is presented by Gowdy & Ulsamer (*Am. J. Dis. Child.* 1976, **130**, 247). Vacuolation of the brain was found in 18% (14 out of 76) of the autopsy samples of an exposed group—infants dying in the neonatal period in hospitals routinely employing HCP bathing—but also in two (11%) of the 18 samples taken from similar institutes not using the bacteriostat for neonatal bathing. In a letter to the *British Medical Journal* (1977, **1**, 904), Young & Treadway cited this result—that there was no statistical difference in the incidence of vacuolation in the HCP-exposed and non-exposed group—as evidence that HCP was not hazardous to the newborn. However, Gowdy (*ibid* 1977, **2**, 1353) did not think this interpretation was justified. He stressed that the very small size of his control group would make it very difficult to demonstrate a statistically significant difference unfavourable to HCP. It is also worth stating that even in hospitals where the newborn are not bathed directly with HCP, exposure to the bacteriostat can still occur; care must therefore be exercised in choosing a control group. In the study by Gowdy & Ulsamer (*loc. cit.*), for example, HCP was found to be present in the brain tissue of one of the two infants whose mothers were prepared with it just before delivery. Similar placental transfer of HCP has been demonstrated in the rat; measurable amounts of HCP were detected in the foetus following the treatment of the pregnant animal either orally (10 mg HCP/kg) or dermally (8 mg/kg) (Kennedy *et al. Toxic. appl. Pharmac.* 1977, **40**, 571).

In spite of histological evidence that HCP does exert a neurotoxic effect on the neonatal brain, it is still uncertain whether such changes manifest themselves in any clinical signs of toxicity. Shuman *et al.* (*loc. cit.*) noted that there were no distinct clinical characteristics in the admittedly small number of infants in his study who were subsequently shown to have severe lesions of the CNS. Clinical follow-up by Plueckhahn & Collins (*loc. cit.*) of a larger number of premature infants bathed frequently with HCP produced similar conclusions. It was calculated that over half of the 872 low birth-weight infants, who had undergone four or more HCP washings but who were discharged from hospital fit and well, were likely to have suffered brain vacuolation. This group

included four sets of live-born twins in which one sibling from each set had died before discharge and showed vacuolation at autopsy. As the four survivors had undergone similar HCP washing it was highly probable that they too had developed CNS vacuolation. Clinical follow-up for 2 to 12 years could identify no abnormalities in mental or physical development. The progress of three sets of triplets with hyperbilirubinaemia and weighing less than 1·725 kg at birth was also followed. Each of the eight infants eventually discharged had been given a whole-body HCP wash on alternate days throughout the hospital stay from 6 to 12 weeks and therefore almost certainly suffered from CNS vacuolation. Observation for a period of over 10 years identified no particular health or developmental problems. One possible instance where HCP may have produced clinical signs of toxicity was reported by Tyrala *et al.* (*J. Pediat.* 1977, **91**, 481). An infant whose blood-HCP concentration reached 4·35 μg/ml after seven baths in 3% HCP suffered a rapid increase in head circumference during the bathing period, and shortly after developed jaundice, rickets and pneumonia. HCP-induced cerebral oedema could not, in the authors' opinion, be ruled out as the cause of these developments.

The possibility that death may be a clinical effect of HCP-induced vacuolation was investigated in the study by Plueckhahn & Collins (*loc. cit.*). Two independent pathologists evaluated the plausibility of the recorded causes of death of the 267 premature infants who weighed less than 2 kg at birth. There was no variation between the plausibility of the recorded causes of death in those infants with vacuolation and in those without. By contrast, in the 46 infants considered by Shuman *et al.* (*loc. cit.*) there was significant relation between the brain lesion and the absence of other anatomically apparent cause of death, implying that vacuolation itself may have been the cause of death in some infants.

While whole-body bathing of neonates with 3% HCP solution has been practised in some hospital nurseries for almost 20 years, there is still disagreement as to what blood level of HCP is produced by this regime. Some years ago, a small study indicated that levels up to 0·65 μg HCP/ml blood, approaching those known to cause brain lesions in animals, followed from the routine use of 3% HCP (*Cited in F.C.T.* 1972, **10**, 114). These results have, however, been criticized on the grounds that the site where the blood was taken may have been contaminated with the bacteriostat. More recent work by McQueen & Ferry (*Br. med. J.* 1977, **1**, 637) would seem to be immune from similar criticism, as blood was collected by umbilical arterial catheter. Levels up to 0·61 μg HCP/ml from repeated application of HCP to the skin and up to 0·50 μg/ml after a single application on the day of delivery were found in full-term infants. Estimations of HCP levels following the single application of 5 ml to three premature infants were 1·18, 1·37 and 0·75 μg/ml whereas application of 15 ml of HCP resulted in a peak blood-HCP level of at least 2·28 μg/ml.

Similar results were reported by Tyrala *et al.* (*loc. cit.*). Premature infants of average weight 1·48 kg were washed with soap containing 3% HCP for 3 days and then for 9 days with the 3% HCP soap diluted 1:1 with water (Group I). A second group (Group II) was washed for 6 days with the diluted soap and for the remaining 6 days with a 3% HCP soap solution. The level of HCP in the blood followed first-order kinetics with a half-life of from 6 to 44 hours, peak levels occurring 6 to 10 hours after bathing. In Group I, HCP was present in the blood at a concentration of from 0·148 to 2·775 μg/ml after nine baths; five of the six infants with a weight of less than 1·20 kg had blood levels greater than 1·0 μg/ml. In Group II, HCP blood levels ranged from 0·15 to 4·35 μg/ml 1 day after the seventh bath; all eight infants weighing less than 1·1 kg had HCP levels in excess of 1 μg/ml. Skin condition was also shown to be important in determining the extent of absorption of the bacteriostat. The infants in Group I suffering from skin problems had average HCP-blood levels of 1·615 μg/ml whereas an average level of 0·609 μg/ml occurred in the subgroup with normal skin.

As toxicity is dependent on dose, the suggestion of Corner and her colleagues at Bristol Maternity Hospital that dusting powder containing 0·33% HCP could replace the traditional 3% HCP solution warrants consideration' (Corner *et al. Br. med. J.* 1977, **1**, 636). Apparently the powder has been used at Bristol since 1958 and has proved adequate in reducing staphylococcal colonization and disease. When the powder was used on mature babies, blood HCP levels were well below the presumed toxic levels, as they were even for low birth-weight infants treated for up to 49 days.

Conflicting evidence for the efficiency of a "reduced" dose of HCP, at least when present in solution, comes from the study of Hyams *et al.* (*loc. cit.*). Moreover a 1·5% HCP solution proved to have inadequate bacteriostat properties when tested by Tyrala *et al.* (*loc. cit.*). Three of the 27 infants bathed for 6 days with this HCP concentration became colonized, and it was necessary to increase the strength of the HCP solution to the traditional 3% level in order to maintain microbiological sterility.

Comment

The result of the 1972 FDA action on HCP was to place the responsibility for the continued use of this material on the physician. There is now strong evidence to suggest that brain lesions commonly occur in premature infants bathed regularly with HCP. At present, it does not seem that HCP presents any hazard to full-term babies unless, perhaps, they suffer from skin problems. As the benefits that follow from the use of HCP in the nursery are by no means clearly defined, it seems only reasonable that the decision to use HCP in any particular circumstance should reside in the hands of an expert. So far, at least, the action of the FDA in this case has stood the test of time.

[J. Hopkins—BIBRA]

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TOXICOLOGY: BIBRA ABSTRACTS AND COMMENTS

COLOURING MATTERS

The fate of curcumin

Wahlström, B. & Blennow, G. (1978). A study on the fate of curcumin in the rat. *Acta pharmac. tox.* **43**, 86.

Turmeric is toxic to mammalian cells *in vitro* (Goodpasture & Arrighi, *Fd Cosmet. Toxicol.* 1976, **14**, 9) but the relevance of this finding to ingestion by man is unclear. On the basis of feeding studies in the rat and dog, the Joint FAO/WHO Expert Committee on Food Additives recommended temporary ADIs (Acceptable Daily Intakes) of up to 2·5 mg/kg for turmeric and 0·1 mg/kg for curcumin (present in turmeric at about 3%), but further feeding studies were required by 1980 (*Cited in F.C.T.* 1975, **13**, 663; *ibid* 1979, **17**, 397). It has now been demonstrated that curcumin is only poorly absorbed from the gut, and is rapidly metabolized and excreted.

Rats given 1–5 g curcumin/kg in arachis oil by gavage displayed no apparent effects, and relative heart, liver and kidney weights were unchanged. Of a single oral dose of 1 g/kg, some 67–87% was excreted unchanged in the faeces over 3 days, mostly in the first 48 hr, and negligible amounts (0·0054% of the dose, or less) appeared in the urine. Biliary levels of curcumin rose to a steady level of about 1 μg/ml after 30 min, and after 3 hr only 0·00056% of the dose had been eliminated by this route. About 0·015% of the administered curcumin was present in the liver, kidneys and body fat after the same period, but plasma levels remained close to zero throughout.

After iv injection of curcumin, the bile contained appreciable amounts within 5 min, and a peak level of about 0·2 mg/ml was attained in 30 min. On average, 2·1% (about 20% of which was in conjugated form) was excreted in the bile (against a massive concentration gradient) within 3 hr, and a further 3·1% of the dose was found in the liver and 0·5% in the kidneys after this time. Plasma levels were reduced by a factor of 100 or more within the first 5–10 min, and were close to zero after an hour. A transient increase in bile flow was observed after the injection, and this was demonstrated again in liver-perfusion studies. As much as 10% of a perfused dose appeared in the bile over 3 hr, and some 49% of this was in conjugated form. In microsome or hepatocyte suspensions, 60–95% of added curcumin had disappeared within 30 min; the rate was dependent on the temperature, and the possibility that the removal of curcumin was due to microsomal binding was eliminated.

The authors also briefly report that a long-term, three-generation rat study on curcumin failed to reveal any adverse reproductive or carcinogenic effects (N. Ghatak, personal communication). The poor absorption and rapid metabolism and excretion of curcumin seem likely to be responsible for this lack of effect in the intact animal.

FLAVOURINGS, SOLVENTS AND SWEETENERS

Mutagenic safrole

Green, N. R. & Savage, J. R. (1978). Screening of safrole, eugenol, their ninhydrin positive metabolites and selected secondary amines for potential mutagenicity. *Mutation Res.* **57**, 115.

Investigations of safrole (4-allyl-1,2-methylenedioxybenzene) have brought to light a large number of metabolites, several of which react electrophilically with nucleosides in a manner characteristic of known carcinogens (*Cited in F.C.T.* 1978, **16**, 294). Eugenol has a chemical structure similar to that of safrole, but has been less extensively tested.

The present study evaluated the mutagenicity of safrole, eugenol and several of their metabolites in two assay systems. The metabolites tested were safrole metabolites I (3-*N*,*N*-dimethylamino-1-(3′,4′-methylenedioxyphenyl)-1-propanone), II (3-piperidyl-1-(3′,4′-methylenedioxyphenyl)-1-propanone) and III (3-pyrrolidinyl-1-(3′,4′-methylenedioxyphenyl)-1-propanone), and eugenol metabolites II (3-piperidyl-1-(3′-methoxy-4′-hydroxyphenyl)-1-propanone) and III (3-pyrrolidinyl-1-(3′-methoxy-4′-hydroxyphenyl)-1-propanone). The secondary amines, dimethylamine, piperidine, pipecolic acid, proline and pyrrolidine were also tested. Reaction of allyl ketone metabolites of safrole and eugenol with secondary amines is a possible source of the ninhydrin-positive metabolites excreted in the urine and identified as tertiary aminopropiophenones.

Four mutant strains of *Salmonella typhimurium* were used in the direct bacterial assay. Safrole and safrole metabolite II caused a statistically significant increase in the number of revertant colonies in strains TA1530 and TA1532, but did not achieve the tenfold increase in spontaneous reversion rate or mutant frequency that has been suggested as the criterion for classification as 'mutagenic'. In the presence of an activation system, safrole at 0·0025 M and safrole metabolite II at 0·015 M concentrations were mutagenic in these two strains but were not as active as nitrosodimethylamine (NDMA), the positive control. Dimethylamine was mutagenic against strain TA1530 at 0·05–0·5 M concentrations in this microsomal assay system, but none of the other secondary amines gave positive results.

In the host-mediated assay, young male mice were

given ip injections of *S. typhimurium* followed by an intramuscular injection of one of the test compounds. Subsequently, fluid from the peritoneum was incubated and the mutant frequency was determined. Safrole and safrole metabolite II were mutagenic in strains TA1950 and TA1952, although again they exhibited lower activity than NDMA, the positive control. The other two safrole metabolites, and eugenol and its metabolites were not found to be active.

This study shows safrole and safrole metabolite II to be weak mutagens both *in vitro* and *in vivo*. Safrole had previously been identified as a weak liver carcinogen in the rat but its mutagenicity had not previously been demonstrated.

ANTIOXIDANTS

Tartaric acid metabolism

Chasseaud, L. F., Down, W. H. & Kirkpatrick, D. (1977). Absorption and biotransformation of L(+)-tartaric acid in rats. *Experientia* **33**, 998.

Chadwick, V. S., Vince, A., Killingley, M. & Wrong, O. M. (1978). The metabolism of tartrate in man and the rat. *Clin. Sci. mol. Med.* **54**, 273.

L(+)-Tartaric acid occurs naturally in many fruits and is produced as a by-product in wine making. The acid and its salts are used in the food industry in a wide variety of products such as baking powder, soft drinks and confectionery. Studies of tartrate metabolism have shown species variations. Following oral administration of tartrate, rats excreted 73% of the dose unchanged in the urine (Gry & Larsen, *Arch. Tox.* 1978, **36**, Suppl. I, 351) but humans excreted only 17% in the urine (Finkle, *J. biol. Chem.* 1933, **100**, 349). However in the latter study after parenteral administration to man, tartaric acid was recovered almost quantitatively in the urine, and it has been suggested that the differences in urinary excretion of tartaric acid following oral and parenteral administration are due to the metabolism of the compound in the intestine. It has been shown that tartaric acid can be metabolized by gut flora (*Cited in F.C.T.* 1967, **5**, 256).

The absorption and metabolism of tartrates in man and rats have now been investigated using radioactive-labelling techniques. In the first study cited above, 400 mg monosodium L(+)-[^{14}C]tartrate/kg was administered to rats either by oral intubation or iv injection. After oral administration to three male and three female rats the amounts of ^{14}C excreted in the urine, faeces and expired air within 48 hr were 70·1, 13·6 and 15·6% respectively. The corresponding figures for rats dosed iv were 81·8, 0·9 and 7·5%. These results indicate that the oral dose of L(+)-tartrate was absorbed and that significant parts of both oral and parenteral doses were metabolized to $^{14}CO_2$ in the body tissues.

In the second study cited, monosodium DL-[^{14}C]-tartrate was given orally and ip to man and to rats, and by direct injection into the caecum of rats. The rats were administered a dose of 20 μCi sodium DL-[^{14}C]tartrate with 18·8 mg L(+)-tartrate/kg as carrier by all three routes. In man, five subjects were given an oral dose of 5 μCi sodium DL-[^{14}C]tartrate with 2·5, 5 or 10 g sodium L(+)-tartrate as carrier and one subject received 10 μCi DL-[^{14}C]tartrate with 125 mg sodium L(+)-tartrate as carrier by the iv route.

In rats, 63% of the sodium [^{14}C]tartrate dose was excreted unchanged in the urine within 24 hr of ip administration and 9% was excreted as $^{14}CO_2$ within 6 hr. After oral administration only 51% was excreted in the urine but more (21%) ^{14}C appeared in expired air as $^{14}CO_2$. Following caecal injection, 67% ^{14}C was expired as $^{14}CO_2$ and less than 2% was excreted in the urine. In man, the percentage of ^{14}C recovered in the breath and urine seemed to be independent of the tartrate dosage level when the tartrate was given orally to five subjects. The majority of ^{14}C was released in the breath (46%) and only 12% of the dose was excreted unchanged in the urine. Following iv administration of tartrate to one subject only, 64% of the dose was excreted in the urine over 22 hr and 18% was excreted in the breath as $^{14}CO_2$ over 8 hr.

The results indicate that the major site of tartrate metabolism is the intestine, although the liberation of $^{14}CO_2$ following parenteral administration does indicate some metabolism by body tissues. Several genera of intestinal bacteria were shown to liberate $^{14}CO_2$ from labelled tartrate in tartrate broth, and when grown on faecal incubates the bacteria metabolized sodium L(+)-tartrate more rapidly than sodium D(−)-tartrate. When 1·5 mmol sodium L(+)-tartrate/kg/day was given orally to two human subjects, the tartrate raised the pH of the urine and the reduction in urinary H^+ ion excretion indicated that an average of 84% of the dose was metabolized. No evidence of renal toxicity, in terms of creatinine clearance or proteinuria, was found.

The results of both studies indicated that some metabolism of tartrate occurred in body tissues. Chasseaud *et al.* (*loc. cit.*) conclude that in the rat oral doses of L(+)-tartrate are extensively absorbed, but the authors of the second paper cited consider that the major source of $^{14}CO_2$ was from bicarbonate or a bicarbonate precursor produced by bacteria in the gut equilibrating with CO_2 in the blood.

[It should be noted that in the experiments described above, radioactively labelled L(+)-tartrate was used in the one case and labelled DL-tartrate in the other. Previous studies have shown that L(+)-tartrate and synthetic racemic DL-tartrate are not metabolized in the same manner (*Cited in F.C.T.* 1978, **16**, 498).]

PRESERVATIVES

Predicting urethane levels in beverages

Solymosy, F., Antoni, F. & Fedorcsák, I. (1978). On the amounts of urethane formed in diethyl pyrocarbonate treated beverages. *J. agric. Fd Chem.* **26**, 500.

Diethyl pyrocarbonate (DEPC) has been used as a preservative for wines, beers and soft drinks. However, DEPC treatment results in increases in the urethane content of the treated beverages, although the extent of these increases has been disputed. Some workers have reported relatively high levels of urethane formation (*Cited in F.C.T.* 1972, **10**, 588) but others claim that much lower amounts of urethane are produced (Ough, *J. agric. Fd Chem.* 1976, **24**, 328). The urethane arises from reaction of ammonia with DEPC and the amount of urethane produced is related to the pH and ammonium ion concentration of the beverage (*Cited in F.C.T.* 1972, **10**, 588). Since urethane is a known carcinogen in several animal species (*ibid* 1978, **16**, 87) limits on the use of DEPC have been imposed (*ibid* 1974, **12**, 407). In the paper cited above the authors derive equations from reaction kinetic calculations which can be used to predict the amount of urethane that will be formed in a beverage, of known ammonium ion concentration and pH, treated with DEPC.

Acetate buffer, containing 10% w/v ethanol, or wine was shaken for 48 hr with different amounts of DEPC under varying conditions of pH and temperature. The ammonia content was determined before and after DEPC treatment, and the difference in ammonia content was taken to be equivalent to the amount of urethane formed. Theoretical values for urethane production were calculated using reaction rate constants for the hydrolysis of DEPC in acetate buffer and in acetate buffer containing ethanol and for the reaction of DEPC with ammonia.

Comparisons of the experimental and theoretical values of urethane production showed that the calculated values were almost equivalent to the amounts of urethane found in DEPC-treated buffer and ethanol but overestimated the amount determined in wine.

Under the conditions of acidic pH and low concentrations of DEPC that occur in beverages, the concentration of ammonia can be considered constant throughout the reaction and the authors derive the following equation:

$$[\text{Urethane}] = k_2[NH_3 + NH_4^+]b\frac{[\text{DEPC}]}{k'}$$

where square brackets indicate the concentrations of the compounds, k_2 is the second-order rate constant for the reaction of DEPC with ammonia, k' is the pseudo-first-order rate constant for the hydrolysis of DEPC, and b = antilog (pH − pK). The pK_a values for ammonia were reduced by 1·3% when applied to wine or aqueous solutions containing 10% (w/v) ethanol.

Using this equation, expected values for urethane concentration were calculated from data in the literature. In general these theoretical values were in good agreement with the reported experimental values.

The authors claim that using this method of calculation it can be predicted that if an average wine (pH 3·44, ammonia 0·205 mM) is treated with 300 ppm DEPC, the amount of urethane produced will not exceed 10 μg/litre. They argue that the use of DEPC in beverages could be controlled by determining the pH and ammonium content of the beverage and calculating the expected amount of urethane that would be produced if DEPC were added.

[Presumably allowance should be made for urethane already present in the beverage. Urethane has been shown to occur naturally in fermented foods and beverages, e.g. 1·3–4·9 μg/litre in wine (Ough, *J. agric. Fd Chem.* 1976, **24**, 323). Addition of DEPC could therefore take the level of urethane above 10 μg/litre—the maximum level considered by the Joint FAO/WHO Expert Committee on Food Additives to be acceptable in soft drinks (*Cited in F.C.T.* 1974, **12**, 407).]

Nitrosamines in human faeces

Wang, T., Kakizoe, T., Dion, P., Furrer, R., Varghese, A. J. & Bruce, W. R. (1978). Volatile nitrosamines in normal human faeces. *Nature, Lond.* **276**, 280.

The formation of nitrosamines by bacterial action has been demonstrated in a variety of organs and body fluids, including the small and large intestines of the rat (Klubes *et al. Fd Cosmet. Toxicol.* 1972, **10**, 757) and the gastric juice and saliva of man (*Cited in F.C.T.* 1978, **16**, 627; *ibid* 1979, **17**, 92). It has now been shown that nitrosamines are also present in normal human faeces.

In a preliminary analysis of extracts of freeze-dried human faeces, *N*-nitrosodimethylamine (NDMA), *N*-nitrosodiethylamine (NDEA), *N*-nitrosodibutylamine (NDBA), *N*-nitrosopiperidine (NPIP) and *N*-nitrosomorpholine (NMOR) were tentatively identified. Faecal samples were then collected from 12 healthy volunteers at the Ontario Cancer Institute and were immediately extracted with dichloromethane, after which the extract was dried, concentrated, distilled, re-extracted and subjected to gas or high-pressure liquid chromatography combined with thermal energy analysis. Recovery of intentionally-added nitrosamines by this method ranged from 51% for NDMA to 88% for nitrosopyrrolidine (NPYR) and NMOR. The chemicals used in the procedure contained no volatile nitrosamines, and addition of ascorbic acid to the faeces, at a concentration sufficient to inhibit nitrosation of amines, did not affect the yield.

NDMA was detected in samples from 11 of the 12 donors, at levels of 0·2–1·5 μg/kg, NDEA was found in eight samples at levels of 0·2–13 μg/kg (two samples, one from each sex, exceeded 10 μg/kg), and NDBA, NPYR and NMOR occurred, respectively, at levels up to 0·9, 0·8 and 1·3 μg/kg in the faeces from two, three and five donors. These levels were much higher than those in the freeze-dried samples, suggest-

ing that substantial losses occurred during the freeze-drying process, but were similar to those found in some foods (*ibid* 1978, **16,** 627). However, it is suggested that faecal nitrosamines could well pose a greater hazard than those in food, due to the long, continuous nature of the exposure.

AGRICULTURAL CHEMICALS

On the track of ethylene thiourea

Ruddick, J. A., Newsome, W. H. & Iverson, F. (1977). A comparison of the distribution, metabolism and excretion of ethylenethiourea in the pregnant mouse and rat. *Teratology* **16,** 159.

Allen, J. R., Van Miller, J. P. & Seymour, J. L. (1978). Absorption, tissue distribution and excretion of ^{14}C ethylenethiourea by the rhesus monkey and rat. *Res. Commun. chem. Path. Pharmac.* **20,** 109.

Ethylene thiourea (ETU), a decomposition product of ethylene-bis(dithiocarbamate) fungicides, has been shown to be mutagenic in bacteria and teratogenic in rats (*Cited in F.C.T.* 1977, **15,** 361; *ibid* 1979, **17,** 177). Its transplacental passage into the foetus has been demonstrated (*ibid* 1977, **15,** 80).

The first study cited above describes the intragastric treatment of rats and mice with 240 mg ETU/kg on day 15 of gestation. The compound was labelled with ^{35}S for rats and with ^{14}C for mice. Animals were killed 3, 6, 12, 24 and 48 hr after dosing, and samples of maternal liver, kidney, gastrocnemius muscle and blood, the placenta and whole foetuses were examined. Urine and faeces were collected for the periods 0–6, 6–12, 12–24 and 24–48 hr.

The concentrations of ETU in mother and foetus 3 hr after dosing were similar in both species. At 6 and 12 hr, mouse tissues contained about one half and one sixth, respectively, of the tissue concentrations in the rats, but by 24 hr tissue levels of ETU were again similar in the two species. At 48 hr, radioactivity was detectable in all rat tissues but only in the liver of the mouse. Excretion of ETU was faster in the urine of mice than of rats, but total urinary excretion by 48 hr was similar, with totals of 73·5% of the dose for mice and 70·5% for rats. Faecal excretion amounts to 2–3% of the dose in both species. The peak activity in the blood was reached at the same time (1·3–1·4 hr) in the rat and mouse, but the peak concentrations (214·6 and 136·7 μg/g, respectively) differed significantly. The calculated half-life of ETU in maternal blood was 5·5 hr for mice and 9·4 hr for rats. The dominant compound in rat urine was ETU and there was one minor metabolite; in mouse urine, ETU and two major metabolites appeared.

Although foetal levels of radioactivity were similar in both species at 3 hr and were also similar to the levels in maternal tissues and the placenta, clearance of activity from the foetus was much faster in the mouse than in the rat. By 12 hr, activity expressed as ETU had fallen from the 3-hr value of 175·6 μg/g to 10·6 μg/g, while the corresponding fall in the rat was from 152·6 to 76·2 μg/g. These findings may explain the greater susceptibility of the rat than the mouse to the teratogenic effects of ETU.

In the second report cited, two female rhesus monkeys were given ^{14}C-labelled ETU in an intragastric dose of 40 mg/kg and their urine and faeces were collected over 48 hr. Total urinary excretion of the label over that period was 47 and 64%, the faecal excretion was 0·45 and 0·63% of the dose for the two animals. Tissue concentrations of radioactivity were fairly low and were similar in all the organs and tissues analysed. The main contributors to the total body burden (21 and 28% of the dose at 48 hr) were therefore the muscle, skin and blood. No gross or microscopic changes were seen in the tissues.

In four rats given the same dose of labelled ETU, urinary excretion averaged 81·9% and faecal excretion 1·32%. In contrast to the situation in the monkey, less than 1% of the activity remained in the tissues after 48 hr, and the highest level was retained in the liver (0·013% of dose/g tissue). Other tissues except the thyroid had an average concentration of 0·004%/g. In two rats, thyroid concentrations of 0·91 and 1·07%/g were recorded. Again, no gross or microscopic changes were seen in the tissues.

Although the authors suggest that the inconsistency in the levels of radioactivity found in the rat thyroids was probably due to an analytical problem, accumulation of ETU in the thyroid gland has been demonstrated and has been held responsible for the adverse effects of ETU on the rat thyroid. This study provided no evidence of any comparable affinity of the monkey thyroid for ETU.

PROCESSING AND PACKAGING CONTAMINANTS

Lysinoalanine in the neonatal rat

Struthers, B. J., Hopkins, D. T., Prescher, E. E. & Dahlgren, R. R. (1978). Effects of protein-bound lysinoalanine, N^{ϵ}-DL-(2-amino-2-carboxyethyl)-L-lysine on fetal and neonatal rats. *J. Nutr.* **108,** 954.

Lysinoalanine (LAL), which occurs in proteinaceous foodstuffs that have been subjected to alkali treatment, has produced karyomegalic changes and necrosis in the renal tubular cells of rats (Woodward & Short, *Fd Cosmet. Toxicol.* 1977, **15,** 117). In a subsequent comparative study of the response of weanling Sprague–Dawley and Wistar rats to a diet containing alkali-treated soya-bean protein with a 1% LAL content (*Cited in F.C.T.* 1978, **16,** 499) marked renal-cell cytomegaly occurred only in the Sprague–Dawley rats, although renal calcification was more severe in the Wistar strain. The rats used in the reproduction and teratology study cited above were of the Sprague–Dawley strain.

The female rats were fed throughout gestation and lactation on diets containing a total of 30% protein, of which 5–30% was an alkali-treated soya-bean protein isolate with a LAL content of 1%, the balance being a soya-bean assay protein free of LAL. The LAL levels of the four test diets was therefore 500, 1000, 2000 and 3000 ppm.

No skeletal or organ abnormalities were found in the foetuses from the treated dams and there was no cytomegaly in the foetal kidneys. Renal cytomegaly was apparent, however, 30 days after birth in pups born to rats on the 3000-ppm LAL diet. There were no significant differences from the controls in numbers of implantations, corpora lutea or resorption sites, live births per litter, total pups per litter, postnatal deaths or birth weights in the LAL-fed groups. However, pups from dams fed 2000 or 3000 ppm LAL gained weight more slowly than the others. No LAL was detected in the maternal milk, and this failure to gain weight normally was ascribed to a decrease in milk production resulting from a reduction in the availability of the protein in diets containing a high proportion of alkali-treated soya-bean product.

THE CHEMICAL ENVIRONMENT

Chromium and the foetus

Gale T. F. (1978). Embryotoxic effects of chromium trioxide in hamsters. *Envir. Res.* **16,** 101.

Studies on the toxicity of chromium and its compounds have resulted in reports of damage to the integument, liver, kidney and respiratory system in both man and experimental animals (Baetjer *et al. Chromium*; National Academy of Sciences, Washington, DC, 1974). There is also evidence to indicate a risk of broncho-pulmonary carcinoma for individuals handling chromium compounds in industry (*Cited in F.C.T.* 1976, **14,** 215). Despite the fairly extensive work on the toxicology of these materials, studies of their effects on the developing mammalian embryo appear to have been neglected. One contribution on this aspect, however, appeared recently.

Hexavalent chromium in the form of the trioxide was administered iv to pregnant golden hamsters in a dose of 5, 7·5, 10 or 15 mg/kg on day 8 of gestation. The animals were observed for toxic effects until they were killed on day 12, 14 or 15 of gestation. All foetuses were examined for external malformations and a proportion for skeletal and internal abnormalities. Resorption sites in the maternal animals were counted, and stained sections of maternal gall bladder, liver and kidney were examined to detect possible histopathological changes.

Of the hamsters given the highest dose, 75% died shortly after treatment. Those given 5 mg/kg showed no ill effects, but the two intermediate doses caused weight loss, changes in urine colour, mottling of the kidneys, distended gall bladders and histological changes in the liver and kidneys.

Exposure to the chromium trioxide killed foetuses *in utero*, the severity of the observed effects being directly related to the dose level. In all cases the resorption sites contained necrotic material unrecognisable as foetal tissue, indicating embryonic deaths early in the gestation period. Abnormal foetuses were retarded or oedematous or displayed specific congenital malformations, in a dose-dependent manner. Only a few external malformations were seen at the lower dose levels but the incidence of cleft palate was significantly higher in all of the experimental groups than the 2% frequency observed in the controls.

Of the internal malformations detected, hydrocephalus was the major one and occurred only in the treated groups. In addition, a few foetuses exposed to chromium exhibited enlargement of the right atrium of the heart and one displayed right renal agenesis. The incidence of supernumary ribs did not show a dose-dependent effect, since the offspring of animals given the 10-mg/kg dose had a lower frequency (7%) of extra ribs than the controls (13%), whilst the experimental groups given the lower doses displayed a frequency at least twice as great as the controls. In all groups, damage to the bones of the cranial vault was minimal, and absence or poor ossification of the arches and bodies of the cervical and caudal regions accounted for most of the vertebral-column defects. Poor ossification was also observed in the manubriae and sternebrae. Skeletal damage of the forelimb was restricted to the 7·5- and 10-mg/kg treatments.

This study represents a preliminary investigation of the teratogenic effects of chromium. Further work is already in progress on the mechanism involved in chromium trioxide-induced cleft palate. The site of action of chromium with the materno-foetal unit—whether it acts directly within the foetus or indirectly by disruption of a metabolic process in the maternal system or placenta—also requires clarification.

Depigmentation by 4-*tert*-butylcatechol

Mansur, J. D., Fukuyama, K., Gellin, G. A. & Epstein, W. L. (1978). Effects of 4-tertiary butyl catechol on tissue cultured melanocytes. *J. invest. Derm.* **70,** 275.

4-*tert*-Butylcatechol (TBC) is used as an antioxidant in several industrial processes, including the manufacture of plastics and polyester resins and the preparation of petroleum products. Topically applied TBC is a depigmenting agent in human and animal skin, although the mechanism of this effect remains unclear (Horio *et al. Int. Archs occup. envir. Hlth* 1977, **39,** 127).

In the above-cited study, the depigmenting effect of TBC on the epidermal cells of guinea-pigs was investigated *in vitro*. Either dimethylsulphoxide (DMSO) or TBC dissolved in DMSO was added to the 5-day-old culture. With concentrations varying from 5×10^{-6} to 3×10^{-5} ml DMSO/ml of medium, the melanocytes did not show any change in shape

or size and behaved similarly to those in the control cultures.

A concentration of $1{\cdot}5 \times 10^{-3}$ mg TBC/ml of medium had little or no effect on about half of the melanocytes over a period of 6 hr, but after only 2 hr about one third of the cells had a ragged contour and their dendrites had narrowed at several sites, presenting a beaded pattern. After treatment for 6 hr many of these affected cells had died. With concentrations greater than 2×10^{-3} mg TBC/ml a large number of the melanocytes died, although the remaining viable cells were structurally normal again within 48–72 hr. In all the experiments, the melanocytes that were still alive 6 hr after the start of treatment were well pigmented.

These results indicate that TBC causes depigmentation by killing the melanocytes rather than by affecting melanogenesis or preformed pigment. This supports earlier reports of an absence of melanocytes from depigmented skin lesions (Kahn, *Archs Derm.* 1970, **102**, 177) and of the persistence of depigmentation for at least 3 yr in a worker exposed to TBC in a polyester-resin plant (Horio *et al. loc. cit.*). The present study suggests that the depigmenting effects of chemicals can be studied *in vitro* and that the technique can be used to demonstrate melanocyte damage similar to that reported *in vivo*.

Butylcellosolve metabolite identified

Jönsson, A.-K. & Steen, G. (1978). n-Butoxyacetic acid, a urinary metabolite from inhaled n-butoxyethanol (butylcellosolve). *Acta pharmac. tox.* **42**, 354.

Urinary metabolites can be useful for monitoring the degree of industrial exposure to a variety of chemicals, a possibility that has received considerable attention, for example, in connection with trichloroethylene (*Cited in F.C.T.* 1978, **16**, 491). A butylcellosolve (*n*-butoxyethanol) metabolite which may prove useful for this purpose has now been identified in rat urine.

The rats were exposed to butylcellosolve at a concentration of 2000 mg/m^3 for 1 hr, and their urine was then collected for 20 hr. Gas chromatography of organic acids in the urine revealed two additional peaks after exposure to butylcellosolve. One of these was due only to a difference in derivation of hippuric acid. The other was consistent with the trimethylsilyl derivative of *n*-butoxyacetic acid, and this was confirmed by its gas-chromatographic retention time and mass spectrum. *n*-Butoxyacetic acid is unlikely to be formed from a large number of other solvents, a fact which adds to its potential value for butylcellosolve-monitoring purposes.

Determining a TLV for 1,2,4-trichlorobenzene

Coate, W. B., Schoenfisch, W. H., Lewis, T. R. & Busey, W. M. (1977). Chronic, inhalation exposure of rats, rabbits, and monkeys to 1,2,4-trichlorobenzene. *Archs envir. Hlth* **32**, 249.

We have previously noted the ability of 1,2,4-trichlorobenzene (TCB) to produce skin irritation (*Cited in F.C.T.* 1976, **14**, 219), and to cause hepatic porphyria on feeding to rats (*ibid* 1965, **3**, 528). In an early inhalation study involving several animal species, the principal target organs were identified as the liver, kidney, brain and mucous membranes (J. Treon, unpublished report 1950). A more detailed investigation of TCB's effects on inhalation has recently been undertaken with the aim of defining a safe level for industrial exposure.

Rats, rabbits and monkeys were exposed to 99·07% pure TCB at nominal concentrations of 25, 50 or 100 ppm for 7 hr/day on 5 day/wk for 26 wk. Tests of pulmonary function and operant behaviour in monkeys, ophthalmoscopic examinations of rabbits and monkeys, and measurements of body weight and haematological and serum biochemical determinations in all species were conducted before and at intervals during exposure. Some rats were killed after 4 or 13 wk and the rest of the animals at wk 26. No exposure-related effects were detected in the rabbits or monkeys, either during exposure or on gross or histopathological examination. However, in rats, transient liver and kidney changes were found at all levels after 4 and 13 wk. In the livers the hepatocytes were enlarged, more vacuolated and individualized, and granuloma formation and biliary hyperplasia were somewhat increased, while in the kidney there was hyaline degeneration in the inner zone of the cortex. As these changes were no longer evident after 26 wk it appeared that they reflected adaptive changes in metabolic processes, but further studies to determine whether other species develop similar transient changes were considered desirable. In the meantime, to provide a margin of safety a TLV of 5 ppm was suggested.

[This recommendation has already been taken up by the ACGIH, in its 1978 TLV booklet a ceiling value of 5 ppm (40 mg/m^3) is listed for TCB (*TLVs. Threshold Limit Values for Chemical Substances and Physical Agents in the Workroom Environment with Intended Changes for 1978*; ACGIH, Cincinnati, OH, 1978). This is intended to prevent mucosal irritation as well as systemic effects.]

A subacute study on turpentine

Savolainen, H. & Pfäffli, P. (1978). Effects of long-term turpentine inhalation on rat brain protein metabolism. *Chemico-Biol. Interactions* **21**, 271.

Turpentine is used in industry and the home as a thinner and general solvent. Despite its long history and known chemical composition, its chronic toxicity is largely unknown. Acute experiments in rats and mice have shown that its action is directed principally against the central nervous system, giving rise to respiratory changes, ataxia, tremors and convulsions (*Cited in F.C.T.* 1967, **5**, 834). Turpentine oil has been established as an allergen (*ibid* 1970, **8**, 238). The above-cited authors have studied the effect of the inhalation of commercial turpentine in a situation that simulated subacute exposure at work and have attempted to correlate the biochemical changes in the brain with the systemic dose.

Forty rats were used as controls and a further 40 were exposed to turpentine, containing 95% α-pinene, in an atmospheric concentration of 300 ppm (12 μmol/litre) for 6 hr/day on 5 days/wk for 8 wk.

Groups of test and control rats were killed at approximately weekly intervals, and brain, blood and perinephric fat samples were taken.

There were no appreciable differences in behaviour or in the acid-proteinase activity in the two groups, but exposure to turpentine caused an accumulation of α-pinene in the perinephric fat and brain. There was little difference during the experiment in the brain level of pinene, which was less than 10% of that seen in the perinephric fat. The protein content of the brain was unaffected by treatment and although the brain level of RNA was lower in the exposed group after 1 and 2 wk, this returned to the control level and even increased toward wk 8. Serum non-specific cholinesterase activity had decreased after 2 wk but returned to normal by wk 4. Serum creatine-kinase activity was similar in the two groups and the brain-enzyme determinations showed no changes during the observation period.

The exposure level of 12 μmol/litre used in this study was one seventh of the LC_{50} for a 6-hr exposure and was three times higher than the present threshold limit value of 100 ppm. The authors found that turpentine accumulated in the mammalian body after these moderate exposures and transient effects were recorded on the RNA content of the brain. No appreciable biochemical effects persisted, however, throughout the 8-wk exposure.

Mutations from allyl chloride

McCoy, E. C., Burrows, L. & Rosenkranz, H. S. (1978). Genetic activity of allyl chloride. *Mutation Res.* **57,** 11.

The National Cancer Institute has reported that mice given allyl chloride (AC) in corn oil by gavage developed forestomach lesions (*Federal Register* 1978, **43,** 50253). It is interesting, therefore, to find that AC is mutagenic *in vitro* when precautions are taken to prevent its evaporation, in contrast to an earlier report of inactivity in a standard Ames test (De Lorenzo *et al. Cancer Res.* 1977, **37,** 1915).

AC's lack of activity in the standard Ames test, in which both bacterial and test agent are incorporated in the agar overlay, was confirmed. However, as this technique involves exposure to molten agar at AC's boiling point of 44–45°C, it seemed possible that AC might have evaporated during the procedure. When AC-impregnated filter discs were laid on the agar plates containing the bacteria (to avoid exposure of AC to agar at 45°C) and the plates were enclosed in a sealed plastics bag (to minimize escape of the vapour) for incubation at 37°C, AC was mutagenic for *Salmonella typhimurium* strains TA100 and TA1535, although not for TA1538. Positive results were also obtained when AC and *S. typhimurium* strain TA1535 were incubated at 37°C for 20 min before the addition of molten agar transfer to an agar plate for re-incubation. The activity of the compound was not enhanced by activated rat-liver microsome preparations. The latter finding and the strains in which a positive result was obtained together indicate that AC is a direct-acting mutagen inducing base-pair substitutions.

When tested in the *Escherichia coli* DNA polymerase-deficient disc test procedure, which like the modified Ames test above did not involve heating to 45°C, AC gave evidence of DNA-modifying activity. It also induced gene conversion in yeast when incubated in a plastics bag at 30°C for 4 hr.

These findings confirm that AC's reported lack of mutagenicity in micro-organisms was due simply to its volatility, and provide an important lesson for the testing of other volatile substances.

Mutagenic petrol

Nylander, P.-O., Olofsson, H., Rasmuson, B. & Svahlin, H. (1978). Mutagenic effects of petrol in *Drosophila melanogaster*. I. Effects of benzene and 1,2-dichloroethane. *Mutation Res.* **57,** 163.

Commercial petrol contains a variety of hydrocarbons as well as a number of aromatic compounds, haloalkanes and lead. Of these components, 1,2-dibromoethane has already been shown to produce sex-linked and autosomal recessive lethals in *Drosophila melanogaster* (Vogel & Chandler, *Experientia* 1974, **30,** 621), and 1,2-dichloroethane has induced base-pair substitutions in *Salmonella typhimurium*, an effect which was enhanced by the presence of a post-mitochondrial liver fraction (Rannug *et al. Chemico-Biol. Interactions*, in press). The latter compound has also induced non-disjunction and recessive sex-linked lethal mutations in *D. melanogaster* (Shakarnis, *Genetika* 1969, **5,** 89). A further component, benzene, is known to produce leukaemia in man (*Cited in F.C.T.* 1977, **15,** 652).

Using a genetically unstable system composed of a transposable genetic element in the X-chromosome of *D. melanogaster*, the reporters of the above-cited study examined the mutagenic effects of petrol (1·0 and 2·5%), benzene (1·0 and 2·0%) and 1,2-dichloroethane (0·1 and 0·5%) given to the larvae in their food supply. Mutagenicity was measured by the frequency of somatic mutations for eye pigmentation. In this system, both the petrol and 1,2-dichloroethane showed mutagenic activity, whilst benzene did not. A strong mutagenic effect was demonstrated with 1,2-dichloroethane. The investigators suggest that this was due to activation with glutathione, mediated by glutathione *S*-transferases, commenting that a synthetic conjugation product, *S*-chloroethylcysteine (between 1,2-dichloroethane and cysteine *S*-2-chloroethylcysteine), has been shown to be more mutagenic than 1,2-dichloroethane in *S. typhimurium* (Rannug *et al. loc. cit.*). They also conclude that the mutagenic activity of the petrol sample was probably due at least in part to its content of dichloroethane.

[The proliferation of positive results for environmental chemicals in mutagenicity tests continues. Whilst it remains important to clarify the mechanisms involved in these mutagenic effects, a comparative assessment of the many tests available and their relevance to possible effects in man is urgently required. Fortunately there is every indication that this need is being recognized.]

The reproductive effects of chloroprene

Culik, R., Kelly, D. P. & Clary, J. J. (1978). Inhalation studies to evaluate the teratogenic and embryotoxic potential of β-chloroprene (2-chlorobutadiene-1,3). *Toxic. appl. Pharmac.* **44,** 81.

β-Chloroprene is a volatile liquid used in large quantities to manufacture neoprene rubber. Several Russian papers have reported that β-chloroprene is embryotoxic, teratogenic and mutagenic to mice and rats even at concentrations below the USSR MAC of 1 ppm. To check these findings, studies have now been undertaken with concentrations up to the US TLV of 25 ppm.

Pregnant rats were exposed to atmospheric levels of 0, 1, 10 or 25 ppm β-chloroprene for 4 hr daily on days 1–12 of gestation to evaluate embryotoxicity, or on days 3–20 to evaluate teratogenicity, and were then killed on days 17 or 21 respectively. Neither study showed any significant effect on the pregnancy rate, the number of litters, the corpora lutea or implantation sites per dam, the live foetuses per litter, the median pre-implantation loss or the median post-implantation loss in litters with resorptions. In the teratology study, the percentage of litters with resorptions was approximately doubled at the two higher exposure levels, but as this increase was statistically significant only at the 10 ppm level, it was regarded as fortuitous. The total number of resorptions was also increased (threefold at 10 ppm and twofold at 1 and 25 ppm), and foetal body weight and foetal length were significantly greater at the highest and the two highest levels, respectively. However, no increase in malformations was seen. In the embryotoxicity study there was no evidence of any exposure-related effects, the number of resorptions being increased slightly only at the 1 ppm level.

When five male rats were exposed to 25 ppm for 4 hr daily for 22 days and then mated with three untreated females weekly for the following 8 wk, there were no effects on the percentage of successful matings, the average number of pups per litter or pup survival.

The β-chloroprene used in these studies was more than 99·9% pure, and contained less than 50 ppm of dimers. It is possible, therefore, that the Soviet findings may have been due to the reaction products (including not only dimers but also peroxides, acids and other substances) that are readily formed in ageing β-chloroprene.

[Although the authors conclude that β-chloroprene is neither embryotoxic not teratogenic at the TLV of 25 ppm, the results of their teratology study suggest that both resorption rate and foetal size were affected to some extent. Moreover, daily exposure was only half the length of a normal working day. It is of interest that this study was among those considered in the NIOSH criteria document on β-chloroprene in 1977, but a reduction of the TLV to 1 ppm was still considered desirable. The ACGIH have also recommended a reduced TLV, of 10 ppm, in their most recent publication (*TLVs. Threshold Limit Values for Chemical Substances and Physical Agents in the Workroom Environment with Intended Changes for 1978*; ACGIH, Cincinnati, OH, 1978).]

The mutagenicity of epoxy resins

Andersen, M., Kiel, P., Larsen, H. & Maxild, J. (1978). Mutagenic action of aromatic epoxy resins. *Nature, Lond.* **276,** 391.

Epoxy resins are one of the most frequent causes of allergy (*Cited in F.C.T.* 1978, **16,** 503). However, there have been no indications, from industrial handling, of systemic effects, and limited animal skin-painting and injection tests some 15–20 yr ago gave no convincing evidence of carcinogenicity. About 90% of such resins are manufactured by condensation of bisphenol A (2,2-bis-(4-hydroxyphenyl)propane; BA) with epichlorhydrin (EC). The latter has been implicated as a mutagen in the Ames test (*ibid* 1978, **16,** 492) and in studies of exposed workers (*ibid* 1978, **14,** 503). An excess of lung cancer deaths among Shell workers has also been tentatively attributed to EC (*Food Chemical News* 1978, **20** (23), 2).

The present study consisted of an Ames test in *Salmonella typhimurium* strains TA100 and TA1535, using BA, EC and three epoxy resins dissolved in dimethylsulphoxide (DMSO). One resin (Epikote 828) is basically the diglycidyl ether of BA, with a small amount of material of higher molecular weight, while the others (Epikotes 1001 and 1004) are formed by condensation of EC with two and four molecules, respectively, of BA (although they also contained some material of lower molecular weight). The resins were all mutagenic to strain TA100, their potency varying inversely with their molecular weight, and EC was also active, but less so than Epikotes 828 and 1001. Mutagenicity was also demonstrated by an aqueous emulsion of Epikote 828 (made ultrasonically), although the resin in this form was less active than in DMSO. With strain TA1535, sensitive like TA100 to mutagens inducing base-pair substitutions, EC was about as potent as with TA100, but the activity of the resins was far lower.

In tests designed to demonstrate the possible mutagenicity of the resin metabolites, incubation of the resins with activated rat-liver microsomes in the presence of NADP (on which the activity of the monooxygenase enzyme system depends) reduced their mutagenic potency in strain TA100 but increased that in TA1535. Mutagenicity in the latter strain was decreased, however, by a rat-liver microsomal preparation lacking NADP. In the latter situation, the epoxide hydrase and glutathione *S*-epoxide transferase enzymes might be expected to be active, with the consequent formation of diols or glutathione conjugates, which seem likely from other studies to be less mutagenic than the corresponding epoxides. Incubation of the resins with a heat-inactivated microsomal preparation confirmed that the decrease in mutagenicity effected by the active preparation in the absence of NADP was dependent on enzyme activity. BA was inactive in both tester strains, whether or not liver enzymes were present.

The findings indicated that the mutagenicity of the resins could not be attributed solely to residues of unreacted EC, and suggested that metabolic conversion could produce a compound that was more mutagenic than the parent compound in some tester strains.

Inhaled methacrylate and the foetus

McLaughlin, R. E., Reger, S. I., Barkalow, J. A., Allen, M. S. & Difazio, C. A. (1978). Methylmethacrylate: A study of teratogenicity and fetal toxicity of the vapor in the mouse. *J. Bone Jt Surg.* **60-A,** 355.

In addition to many other uses, methyl methacrylate (MMA) is used as a cement for fixing prosthetic devices. This involves curing it in the operating theatre and exposing theatre staff to the vapour. Rats exposed for 6 months to MMA, at a concentration only slightly above the TLV of 100 ppm, showed reductions in body and tissue weights and intestinal transit rates and changes in the levels of several serum components (*Cited in F.C.T.* 1977, **15,** 257). Embryotoxic and foetotoxic effects have also been reported in rats given ip injections of MMA and other methacrylates (*ibid* 1973, **11,** 1151). The potential effects of inhaled MMA on the foetus have now been evaluated in mice, using FDA-recommended procedures.

Eighteen pregnant mice were exposed for 2 hr twice daily on days 6–15 of pregnancy to average concentrations of 1330 ppm MMA, obtained by allowing 9 ml of liquid MMA to evaporate in a closed system. The animals were killed on day 18, 2 days before the expected delivery date. The incidence of resorptions and of abnormal foetuses was lower than in controls, and the 0·9% incidence of dead foetuses was not significantly greater than the 0·6% in controls. A slight increase in litter size in the test animals was similarly without statistical significance, but foetal weight was significantly increased. As feed intake was not measured, and no metabolic controls were imposed, the significance of this remained unclear.

Measurement of MMA levels in operating-room air during three total hip replacements revealed a maximum concentration of 280 ppm in the first few minutes after MMA cement mixing was started. The levels fell rapidly, and were below 10 ppm when curing occurred after about 11 min. This study thus provides no evidence to suggest that such use of MMA would increase the risks for operating-room personnel during pregnancy.

NATURAL PRODUCTS

Carrageenan-induced colorectal tumours in rats

Wakabayashi, K., Inagaki, T., Fujimoto, Y. & Fukuda, Y. (1978). Induction by degraded carrageenan of colorectal tumors in rats. *Cancer Lett.* **4,** 171.

The carrageenans are a group of sulphated polysaccharides derived from various seaweeds. Some carrageenans derived from the red seaweed, *Eucheuma spinosum*, have been shown to be ulcerogenic when given orally to guinea-pigs, an effect attributed to the predominance of iota chains in the polymer (*Cited in F.C.T.* 1978, **16,** 294).

In the present study, the carrageenan used was derived from *E. spinosum* and degraded by acid hydrolysis to a sulphate content of about 30% and an average molecular weight of 20,000–40,000. Three groups of Sprague–Dawley rats were fed 1, 5 or 10% of this carrageenan in the diet for up to 24 months. In a second experiment, a 5% solution of the carrageenan was given to rats for 15 months in place of drinking-water, and in a third, rats were given 1 or 5 g/kg body weight in aqueous solution by stomach tube daily for 15 months.

In the first experiment, metaplasia was observed in the colorectal mucosa after 6 months in rats given either the 5 or 10% dietary level, and tumours (squamous-cell carcinomas, adenocarcinomas and adenomas) were seen in both groups after 12 months. The final tumour incidence in the two groups was 20 and 31·7% respectively. In the second experiment, metaplasia appeared after 4 months and was present in all the animals at 15 months; tumours (squamous-cell carcinomas, adenocarcinomas, adenomas and amyosarcomas) first appeared at 10 months and the final incidence was 27·5%. In experiment 3, metaplasia occurred in both groups after 14–15 months, with a final incidence of 36·7 and 100% in the groups on 1 and 5 g/kg, respectively. Animals in the high-dose group developed adenocarcinomas and adenomas from month 15 onwards, the final incidence being 27·6%. No tumours were induced at the lower dose level.

In the first experiment, serial killing of the rats between 6 and 24 months for observation of colorectal changes showed that degraded carrageenan induced first colitis, then metaplasia and finally tumours. In three rats given the carrageenan in the diet and in one that drank the carrageenan solution, a squamous-cell carcinoma metastasized to the retroperitoneal lymph nodes. The incidence of malignancy was notably less after instillation of carrageenan into the stomach than after its intake in the diet.

Bracken sphere of influence widens

Pamukcu, A. M., Ertürk, E., Yalçiner, S., Milli, U. & Bryan, G. T. (1978). Carcinogenic and mutagenic activities of milk from cows fed bracken fern (*Pteridium aquilinum*). *Cancer Res.* **38,** 1556.

Bracken fern is a highly carcinogenic plant that grows in temperate zones of the world. It induces tumours at several sites in a variety of animals (*Cited in F.C.T.* 1978, **16,** 506) although the nature of the bracken carcinogen still remains obscure.

The above-cited study looked at the toxicity, mutagenicity and possible carcinogenicity of milk obtained from cows fed bracken fern. Four cows given a diet of hay contaminated with bracken fern (to give a dose of 1 g bracken/kg/day) all developed papillary tumours of the urinary bladder over a period of 1·5–2 yr. No bladder changes were detected in two control cows fed a normal diet.

Fresh or freeze-dried milk obtained from the treated cows was mixed with a grain diet and fed to two groups of rats. Nine of the 34 rats fed the whole milk and 11 of the 56 fed the powdered milk

developed small intestine, kidney or urinary bladder carcinomas within 117 wk, while no tumours developed in these organs in 70 rats fed on either whole or powdered milk from cows receiving a normal diet or in 20 rats fed a basic grain diet.

Using solvent extraction, six fractions of milk were prepared. Fractions of milk from cows fed bracken induced acute toxic signs of vascular engorgement, petechial haemorrhages of the serous membranes and fatty degeneration of the liver when given ip in a dose of 1·5 g/kg to mice. The diethyl ether and ethyl acetate fractions showed high toxicity; six of the seven mice from each group died. No toxic effects were produced by the corresponding fractions derived from the milk of cows on normal diets. The investigators attempted to assess the carcinogenicity of the six fractions by a pellet-implantation technique. The incidence of bladder carcinomas was significantly greater in the group of mice that received implants of the diethyl ether fraction of milk from bracken-fed cows than in the other implanted and control groups.

Pentane, methanol, chloroform and chloroform–methanol extractions were used to prepare another series of fractions from the milk of the bracken-fed and control cows. These four fractions were tested for mutagenicity against strains TA98 and TA100 of *Salmonella typhimurium*. The chloroform–methanol fraction from the bracken-fed cows demonstrated mutagenic activity for TA100 but not for TA98. No other fractions gave any evidence of mutagenicity.

[The question of the relevance of bacterial tests for mutagenicity, including the Ames test used in this study, is currently receiving much attention, but it may be worth recalling here that bladder implantation is not generally considered to be a reliable indicator of chemical carcinogencity (*Cited in F.C.T.* 1970, **8**, 122; *ibid.* 1973, **11**, 124).]

Monocrotaline effects in sequence

Pleština, R., Stoner, H. B., Jones, G., Butler, W. H. & Mattocks, A. R. (1977). Vascular changes in the lungs of rats after the intravenous injection of pyrrole carbamates. *J. Path.* **121**, 9.

Lalich, J. J., Johnson, W. D., Raczniak, T. J. & Shumaker, R. C. (1977). Fibrin thrombosis in monocrotaline pyrrole-induced cor pulmonale in rats. *Archs Path.* **101**, 69.

Monocrotaline, a pyrrolizidine alkaloid present in *Crotalaria spectabilis*, various Senecio species and other related plants, undergoes conversion to pyrrole derivatives by the microsomal-enzyme systems of the liver (*Cited in F.C.T.* 1974, **12**, 559; *ibid* 1975, **13**, 158). Sublethal doses of monocrotaline pyrrole injected into animals produce hypertensive pulmonary vascular lesions and cor pulmonale, apparently by acting primarily on the endothelium of the pulmonary capillary bed (*ibid* 1975, **13**, 592).

The first paper cited above studies the effect in rats of some synthetic compounds related to monocrotaline pyrrole. No rats died after a single iv injection of 20 mg pyrrole monocarbamate/kg or less, and none showed any lung abnormality 6 wk later. With a dose of 30 mg/kg, death sometimes occurred within a few minutes of injection, the frequency of deaths increasing when the dose was raised to 40 or 60 mg/kg. Death within 24 hr was associated with increasing dyspnoea. Little or no pleural effusion was found in animals dying shortly after injection with pyrrole monocarbamate, but it occurred regularly when death was delayed for several hours.

Pyrrole dicarbamate in a dose of 25 mg/kg caused signs of dyspnoea after 24 hr, and pleural effusion was seen *post mortem*. With a dose of 50 mg/kg, all rats died within 24 hr, showing both pleural effusion and extensive tracheal foaming. Furyl carbamate in a dose of 20 mg/kg or more killed both rats and mice within 24 hr, pleural effusion occurring in the mice but not in the rats. Pyrrole alcohol at 100–200 mg/kg produced only brief narcosis and no gross pathological changes after a 5-wk interval, while 200 mg ethyl carbamate/kg produced no toxic effect or pathological changes in rats. When injected into the carotid artery of rats, the toxic pyrrole carbamates induced oedema in the region of the first capillary bed they reached. Subsequent iv injection of carbon black as a marker showed local deposition, indicating damage, in the post-capillary venules and the capillaries of the lung. Only the compounds with a pyrrole-ring structure and at least one ester side chain had an acute effect on the lung.

The second paper describes an experiment to determine the lung lesions responsible for the development of cor pulmonale in rats given monocrotaline pyrrole. Single sc injections of 25 mg monocrotaline/kg or single iv injections of 2 or 4 mg monocrotaline pyrrole/kg were given, and lung preparations were examined in animals that died or were killed 1–49 days later. All the treated animals examined within 7 days of injection had fibrin thrombi in their lungs. Many of those examined later had developed right ventricular hypertrophy accompanied by varying degrees of alveolar oedema, a proliferation of connective tissue in the alveolar septa, septal cellular hyperplasia and medial hypertrophy of the arterioles. A high proportion of these rats had fibrin thrombi, predominantly in the arterioles and capillaries but also in the pulmonary arteries and veins. Monocrotaline pyrrole was more effective in producing both pulmonary fibrin thrombi and right ventricular hypertrophy than was monocrotaline itself.

From their findings, the authors deduce that injury to the endothelium of the pulmonary vessels by the alkaloid or its derivatives promotes the formation of fibrin and also the development of alveolar septal oedema because of the increased permeability of the injured cells. The resulting sequence of events, including reduction of blood flow through alveolar capillaries occluded by fibrin, leads to pulmonary hypertension and subsequently to right ventricular hypertrophy.

Nutmeg cocktail

Sherry, C. J. & Burnett, R. E. (1978). Enhancement of ethanol-induced sleep by whole oil of nutmeg. *Experientia* **34**, 492.

The hallucinogenic properties of nutmeg have long been known and cases of poisoning have also been

reported (*Cited in F.C.T.* 1964, **1**, 146 & 147; *ibid* 1972, **10**, 437). More recently, a brief monograph on the toxicology of nutmeg oil has been published (Opdyke, *Fd Cosmet. Toxicol.* 1976, **14**, 631). The whole nutmeg, its essential oil and the aromatic constituents of the essential oil (e.g. myristicin and elemicin) have all been examined in relation to these effects. Nevertheless, the mechanism of the action of nutmeg still remains unclear, although it has been suggested that nutmeg, nutmeg oil or myristicin may act as a weak monoamine-oxidase inhibitor (Truitt *et al. Proc. Soc. exp. Biol. Med.* 1963, **112**, 647). Alternative suggestions are that amphetamine derivatives might be formed from myristicin and/or elemicin (Braun & Kalbhen, *Pharmacology* 1973, **9**, 312), or that these components might be transformed into the amino derivatives either by direct transamination or by oxidation and transamination (Shulgin, *Nature, Lond.* 1966, **210**, 380).

To investigate these possibilities further and to clarify reports that alcohol and monoamine-oxidase inhibitors act synergistically, the effect of whole oil of nutmeg (200 mg/kg body weight administered ip) on ethanol-induced sleep was examined in young chicks. For comparison, iproniazid phosphate, known to be a monoamine-oxidase inhibitor was also examined (50, 100, 200 and 400 mg/kg). The dose levels of alcohol by the same route of administration were 1·0, 1·5, 2·0, 2·5, 3·0 and 4·0 g/kg.

The results indicated that when the oil of nutmeg was combined with any dose level of ethanol higher than 1·0 g/kg there was a marked increase in average sleeping time (i.e. average light sleep plus average deep sleep). With increasing dose levels of ethanol, there was an increase in the amount of time spent in deep sleep and in the total sleeping time. When increasing dose levels of the iproniazid phosphate were combined with 3 g ethanol/kg, dose levels up to 400 mg/kg did not cause as large an increase in deep sleep as did 200 mg oil of nutmeg/kg and 1·0 g ethanol/kg.

The authors conclude either that oil of nutmeg is a more potent monoamine-oxidase inhibitor than was anticipated or that the oil has other pharmacological properties. Other data indicating an absence of synergism in the effect of oil of nutmeg with amphetamine suggest a more complex pharmacological profile for the oil.

CANCER RESEARCH

Best of the short cuts in carcinogenic screening

Purchase, I. F. H., Longstaff, E., Ashby, J., Styles, J. A., Anderson, D., Lefevre, P. A. & Westwood, F. R. (1978). An evaluation of 6 short-term tests for detecting organic chemical carcinogens. *Br. J. Cancer* **37**, 873.

Of the hundreds of chemicals evaluated for carcinogenicity by the International Agency for Research on Cancer, only 26 chemicals or manufacturing processes have been deemed to cause cancer in man. Except for arsenic and benzene all these 'human carcinogens' are known to produce cancer in animals. In the case of the aflatoxins, 4-aminobiphenyl, diethylstilboestrol, melphalan, mustard gas and vinyl chloride, evidence of carcinogenicity was obtained first in experimental animals and subsequently in man (from epidemiological and clinical studies). For the majority of chemicals known to be carcinogenic in animals, data in man are limited or non-existent and for practical purposes such chemicals have been regarded as probably carcinogenic to man as well.

Costly and time-consuming lifespan studies, especially in rodents, still serve as the major experimental system for determining the carcinogenic potential of chemicals such as food additives and pesticides, in view of man's lifetime exposure to them. But the deficiencies of this system still prevail and are such that The Royal Society Study Group Report on Long-term toxic Effects (*Cited in F.C.T.* 1979, **17**, 293) concluded that a mass of data on food additives was being obtained from expensive stereotyped analyses and testing procedures in animals and that testing of chemicals was becoming routine and irrational rather than scientific in outlook. More meaningful extrapolation of animal data to man would be facilitated by paying more attention to the mechanisms of toxic action.

It would be impracticable to submit the thousands of industrial and environmental chemicals to long-term carcinogenicity studies, and in the last decade several short-term tests for identifying potential carcinogens have been developed. These are based on the interaction of carcinogens and/or their metabolites with macromolecules, the induction of chromosomal aberrations, mutagenesis, DNA repair and DNA binding. Over 80% of chemical carcinogens are mutagenic and it is now widely held that mutagenicity testing offers a valuable system for screening chemicals before subjecting them to long-term carcinogenicity studies.

Of the short-term (rapid screening) tests available, the Ames test (*ibid* 1975, **13**, 465) and the *in vitro* cell-transformation test (*ibid* 1977, **15**, 483) hold great promise (*Environmental Health Criteria 6: Principles and Methods for Evaluating the Toxicity of Chemicals*; WHO, Geneva, 1978). This view is shared by the above authors, who have undertaken the first comparative blind trial of several tests in the same laboratory.

Some 58 compounds considered to be carcinogens (polycyclic and heterocyclic aromatic hydrocarbons, aromatic amines, alkylating agents including nitrosamines and certain tumour promoters, tumour initiators and compounds positive in bladder-implantation studies) and 62 non-carcinogens were submitted to six tests: the Ames test, mammalian cell transformation in culture, degranulation of rat-liver endoplasmic reticulum, mouse-skin sebaceous-gland suppression, tetrazolium reduction and subcutaneous implantation. Positive results were obtained for 91% of the

carcinogens in both the Ames and cell-transformation tests, but only 37–71% were positive in the other four tests. False positive results (non-carcinogens giving positive results) were low in the cell transformation test (3%), the Ames test (6%) and the implant test (5%) but higher (27–36%) in the other three tests. Whilst combination of the findings of the Ames test and the cell transformation test slightly enhanced the predictive accuracy of identifying carcinogens (detecting all but two of the 58 carcinogens), it also increased the false positives to 9%. Four other tests (transplacental blastomagenesis, piperidine alkylation, the iodine test and the acridine test) were found in preliminary studies to be of poor predictive value and were abandoned.

The authors point out that false negatives (carcinogens giving negative results in the predictive tests) may arise when compounds act via hormonal, solid-state or possibly free-radical mechanisms or via physicochemically-induced tissue damage and its resultant repair.

[Whether a high proportion of compounds found to be positive in the short-term predictive tests ultimately prove to be carcinogens when tested in long-term animal experiments remains to be seen in the years ahead. Meanwhile further development and evaluation of these rapid screening tests should be encouraged. During this evaluation, however, regulatory authorities should resist public pressures for the rejection of chemicals as carcinogens solely on the basis of adverse findings in tests still undergoing assessment, otherwise the continued development of these much-needed screening procedures for carcinogens will be at risk.]

Extrapolating dose–response data: vinyl chloride

Gehring, P. J., Watanabe, P. G. & Park, C. N. (1978). Resolution of dose–response toxicity data for chemicals requiring metabolic activation: Example—vinyl chloride. *Toxic. appl. Pharmac.* **44**, 581.

Methods currently recommended for projecting the dose–response curve of a carcinogen below doses producing an observable effect are based on the dose of chemical administered rather than on the proportion of the dose that gives rise to the response. High doses used experimentally to reveal chronic toxicity may overwhelm the enzymes that activate or deactivate the toxic form of the chemical. In the paper cited above, vinyl chloride (VC) is used as an example to illustrate how the dose-dependent activation of a chemical to its carcinogenic form can be included in extrapolations of the dose–response.

Groups of between three and six male Sprague–Dawley rats were exposed for 6 hr to various concentrations of [^{14}C]VC. The mean concentrations of VC were 1·4, 9·3, 24·7, 51, 109, 250, 511, 1020 and 4600 ppm. Immediately after exposure the rats were killed and the carcasses were analysed for total radioactivity. Previous studies demonstrated that only a small percentage of radioactivity was excreted as metabolites, rather than [^{14}C]VC, in the 72 hr after a 6-hr inhalation exposure. Thus non-volatile radioactivity in the carcass is a good estimate of total metabolized VC.

The metabolism of VC did not increase proportionately with increases in the concentration of VC inhaled. The relationship between the concentrations of metabolites and inhaled VC was established as in keeping with Michaelis–Menten kinetics as described by the equation $v = V_mS/(K_m + S)$, where v is the velocity (μg VC/6 hr), V_m is the maximum velocity (μg VC/6 hr), S is the concentration of VC being inhaled (μg VC/litre) and K_m is the Michaelis constant (μg VC/litre). The values of v and S were given by the experimental results. By transforming the equation to $v = -K_m (v/S) + V_m$, a linear form, it was possible to plot v against v/S, so estimating values for K_m and V_m. Thus toxic effects associated with VC exposure can be related to the amount of VC metabolized rather than to the exposure concentration.

The authors then applied their findings to the results of Maltoni & Lefemine (*Ann. N.Y. Acad. Sci.* 1975, **246**, 195), who reported the evidence of hepatic angiosarcoma in rats exposed to different concentrations of VC for 4 hr/day on 5 days/wk for 12 months. Before applying the Michaelis–Menten equation, using the calculated values for K_m and V_m, it was necessary to adjust V_m for the shorter exposure time ($\times$ 4/6). The values for the velocity of metabolism at different exposure concentrations could then be determined. The known incidence of hepatic angiosarcoma gave a linear probit plot with respect to log v. Extrapolation of this plot allowed projection of the angiosarcoma data below levels of exposure that gave a discernible effect, assuming that the dose–response curve remained valid at such levels.

These workers went on to discuss the use of this approach in assessing the risk to man. This inevitably involved several assumptions: that induction of angiosarcoma is related to the amount of reactive metabolite of VC per unit mass, that exposure of rats for 12 months approximates to exposure of workers for their working lives, that there is no threshold for induction of angiosarcoma in rats or man and that the efficiency of the metabolic processes involved in activating VC is proportional to body-surface area. On these assumptions, V_m for man on a mass equivalent basis is that of rats can be adjusted for the differences in body weight and surface area (SA) of the two species as follows:

$$V_m(\text{man}) = V_m(\text{rat}) \times \text{SA(man)/SA(rat)} \times \text{mass(rat)/mass(man)}.$$

Again, since the exposure period for man would be 8 hr not 6 hr, V_m must be appropriately adjusted. Now the corrected value for V_m and the previously calculated value for K_m can be used in the Michaelis–Menten equation to calculate the amout of VC transformed at given exposure concentrations (S). Using the data from rats on the incidence of angiosarcoma, the theoretical percentage incidence for man is calculated as 1·02% at 200 ppm VC, 0·11% at 50 ppm, $3{\cdot}74 \times 10^{-4}$% at 5 ppm and $1{\cdot}5 \times 10^{-6}$% at 1 ppm. The observed incidence of angiosarcoma in men exposed to more than 200 ppm VC has been reported as 0·02% (Fox & Collier, *Br. J. ind. Med.* 1977, **34**, 1), one fiftieth of the estimated incidence calculated on the basis of the rat data. This discrepancy may

be because people are less sensitive than rats to angiosarcoma or because a practical threshold for the induction of angiosarcoma has been reached. [It may also have resulted from the major assumptions involved in the estimate.]

The authors are aware of the many flaws in these estimates. Nevertheless they emphasize the need to consider pharmaco-kinetic principles both in the design of animal experiments and in the interpretation and extrapolation of results in terms of human exposure.

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VERY ORIGINAL ARTICLE*

MODERN TIMES—IN TOXICOLOGY†

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Our presence in New Orleans this evening brings to mind a little-known association of this area with one of the greatest love stories ever written—the *History of the Chevalier des Grieux and of Manon Lescaut* by the Abbé Prévost. Manon Lescaut was the subject of the opera *Manon* by Massenet (1884) and *Manon Lescaut* by Giacomo Puccini (1893). Banished from their beloved France to the territory of New Orleans, Manon is dying and her lover des Grieux is passionately trying to comfort her. In the libretto to Puccini's opera, Manon is saying, with infinite tenderness ('con passione infinita'):

"Oh! amore! ultimo incanto!
I love you so, I love you
Ah, could I only tell you how much I love you...
Once more to feel your caresses and to drown
in your kisses
Is my final desire!... Ah, I love you."
"Mio dolce amante a me t'appressa... a me!
O stay beside me stay close... stay near me, my
love!"

Well, times change. An appropriate expression of present attitudes is summed up in a girl friend's laconic description of 'Fifty Ways to Leave Your Lover':

"You just slip out the back, Jack
Make a new plan, Stan
You don't need to be coy, Roy
Just get yourself free.
Hop on the bus, Gus
You don't need to discuss much
Just drop off the key, Lee
and get yourself free."

If the world has changed to this extent, should we be surprised that Society's attitude to science and scientists—and more particularly to toxicologists—has undergone an equally radical alteration? Back in the early years of this century the Pure Food and Drug Act of 1906 was enforced by the Board of Food and Drug Inspection (whose first chairman was the redoubtable Harvey W. Wiley) in the Department of Agriculture's Bureau of Chemistry. Wiley's approach to consumer protection was simple and direct. He fed test chemicals to a group of 12 healthy, robust young men from the Agriculture Department, who volunteered to eat all their meals in Wiley's laboratory at the Bureau of Chemistry. Boric acid and borax, salicylates, sulphites, benzoates, formaldehyde, copper sulphate and potassium nitrate were among the items tested in this way. The American people thought this was a great idea and the "Poison Squad" was immortalized in song:

"On prussic acid we break our fast;
we brunch on a morphine stew;
We dine with a match-head consommé,
drink carbolic acid brew;
Corrosive sublimate tones us up like laudanum
ketchup rare,
While tyro-toxicon condiments are wholesome
as mountain air.
Thus all deadlies we double-dare to put us
beneath the sod
We're death immunes and we're proud as
proud—Hooray for the Pizen Squad!"

Today this public gratitude is long forgotten. If anyone in the Pizen Squad was laid low, he died in vain. On the contrary, the vociferous demand for perfect safety has produced an increasing incidence, in some cases reaching epidemic proportions, of two diseases currently ravaging the field of toxicology: pathologophilia and statisticomania. Each alone is a serious condition; occurring together, they are invariably fatal to the successful interpretation of any toxicological study.

In seeking a word to describe the obsessive preoccupation with more and more pathologists, second-guessing each other's diagnostic efforts, I took as my model 'necrophilia', which describes a psychotic fondness for dead bodies often amounting to an erotic attraction for corpses. Appropriate though it might seem by analogy, the term pathologophilia does not express adequately the mental state that desperately grasps after more and more pathologists, like the gambler getting in deeper as he struggles to recoup his losses. Quoting from *Pesticide and Toxic Chemical News*:

"(1) The company pathologists found a tumor incidence that was significant at $P = 0{\cdot}007$; (2) Dr. A for the same dose, $P = 0{\cdot}002$; (3) Dr. B initially found $P = 0{\cdot}073$; and (4) Drs B and C found $P = 0{\cdot}024$."

Interpreting this esoteric information is like the three-card trick: a flick of the wrist—now you see it, now

*The last Very Original Article ("The Health Significance of Environmental Pollution from Biindobioresoate") was contributed by Y. Elwarf (*Fd Cosmet. Toxicol.* 1966, **4**, 565).

†President's Banquet Address, Society of Toxicology, Eighteenth Annual Meeting, New Orleans, LA, 14 March 1979.

you don't. It's almost within your grasp, but keeps eluding you... until, at the end of the line, Dr. Z discovers one more lymphoma and *P* finally comes to rest at the magic number 0·05.

In recent years we have heard much about 'nucleophilic' target sites for chemically-reactive electrophiles. Maybe a large enough spread of pathologists is conceived as a sort of 'pathologophilic' target, like a radar listening device on which all sorts of inspirational messages can zero in.

In what now seems like the remote past, it used to be said of statisticians that they were akin to lavatories—useful when you needed them but undesirable to have in close proximity all the time. All that has changed, the motto now being: the more the merrier. In the matter of FD & C Red No. 40 we have witnessed the manifestations of statisticomania in its purest form with collective nouns of increasing magnitude: from a group of statisticians to a bevy, from a bevy to a covey, from a covey to a pride, from a pride to a phalanx, and from a phalanx to a legion!

But it's not only a question of numbers—statistical methods have also proliferated. Starting with the Bonferroni inequality, we have in quick succession the Cesarone–Giovanardi test for linear trend in proportions, the Spreafico three-tailed exact test and the product limit procedure of Parmeggiani and Tetrazzini. Now the Russians have called for equal time and, in keeping with the obligations of détente, we have adopted the Bolvogorov extensions of Vinogradov's method for testing a dose-related trend in accordance with Belokhvostova–Korosteleva–Kolesnitchenka and have accepted with gratitude the surpassing beauty of Arkadie Ilyitch Khakhlovkin's theorem of statistical inexactitude. As we would say in Georgia: Right on, you'll! Just think of the thrills that still lie ahead, with the advent of Chinese statistics....

These manifestations of statistical *multum in parvo* reached a climax during a marathon session held early this year to decide whether FD & C Red No. 40 accelerated tumour formation in mice. This historic gathering lasted two full days and is now duly entered in the *Guinness Book of Records*, even though no conclusion was actually reached. But why stop at two days? Here's an opportunity for some enterprising government agency to put on 20, or 200 days of continuous statistico-disco—like Rock festivals only better, like a Roman orgy, like the *Thousand and One Nights*. Imagine a modern Scheherazade extolling the abandon, the romance, the debauchery of such statistical ecstasies!

Now we come face to face with the greatest hazard regarding pathogenesis. Once in a rare while, an S plasmid from statisticomania undergoes spontaneous transfer to the causative organism of pathologophilia. This combination is even more dangerous than Ames' strain TA100. In fact, the menace presented by the hybrid organism of statisticopathologomania (known as SPM for short) lies in the possibility that some careless, misguided genetic engineer might go ahead and clone it. Just imagine a race of identical SPM clones swarming through all the government agencies—the toxicologist's life would not be worth living. What we need is a set of guidelines from HEW to protect us from just this sort of recombinant SPM research.

To an increasing degree we are witnessing other similar dangers and frustrations all around us. An example of a fruitless exercise has been the intensive search for ever-diminishing traces of impurities in saccharin, in an effort to account for the alleged carcinogenicity of this sweetener. What a precedent that's going to set! One is reminded of a mythical being, the Booroo-Wooroo bird, much beloved by the British. This creature is reputed to fly around in circles of steadily decreasing diameter until it ultimately vanishes down its own oesophagus. (This, of course, is a sanitized description.) In any event, like a flock of Booroo-Wooroo birds, the search for the vanishing zero has us all in its grip.

Finally, let me refer briefly to the question of affluence. Even for the US, at one time reputedly the most affluent place on earth, the limits of profligate spending have been reached. In the field of medicine, there is general agreement on the need to restrict the continuing increase in hospital and other medical costs before the bills swallow up our Gross National Product. In toxicology, the trend is all in the other direction: more tests, more animals, more dose levels, more facilities, more toxicologists. That would be just fine, were it not for the spectre of fewer and fewer new products being pursued by the accumulated might of a swollen toxicological establishment—rather like the situation in the legal profession of this country today—and see where that has landed us. Toxicologists have an important part to play in protecting the public. We are ready and willing to fulfil this obligation to the utmost of our ability; but we must be allowed to exercise our own professional expertise and our own judgment in choosing the most effective way to discharge our duty and to honour our commitment to the public good. Let us hope for all our sakes, and for the future of our country, that good sense and moderation will prevail before it is too late.

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LETTER TO THE EDITOR

MUTAGENS IN HEAT-PROCESSED MEAT, BAKERY AND CEREAL PRODUCTS

Sir,—The appearance of mutagens and carcinogens in foods during cooking was previously thought to be associated only with high temperature. Lijinsky & Shubik (*Science, N.Y.* 1964, **145,** 53) reported benzo[*a*]pyrene and other polynuclear arenes on the surface of well-done charcoal-broiled steak, apparently due to adsorption of smoke produced when fat from the meat dripped onto burning coals. In later studies Sugimura and colleagues (Nagao *et al.* In *Progress in Genetic Toxicology*, edited by D. Scott, B. A. Bridges and F. H. Sobels; p. 259, Elsevier/North Holland Biomedical Press, 1977) found that pyrolysed proteins and amino acid mixtures contain potent mutagens. The structures of mutagens formed during the pyrolysis (> 300°C) of L-tryptophan (Nagao *et al. loc. cit.*) and L-glutamic acid (Yamamoto *et al. Proc. Japan Acad.* 1978, **54** (ser B), 248) have been published. Recently, Commoner *et al.* (*Science, N.Y.* 1978, **201,** 913; *J. Fd Prot.* 1978, **41,** 996) reported that mutagens (distinguishable from benzo[*a*]pyrene and the pyrolysis products of amino acids) form in hamburger cooked at more moderate temperatures (200°C). We have confirmed this report (Pariza *et al. Cancer Letters* 1979, in press) and have further demonstrated that (1) hamburgers fried at 143°C contained relatively low levels of mutagenic activity whereas hamburgers fried at 191°C or 210°C contained higher mutagen levels, and (2) hamburger also contains heat-stable factors which under some conditions of assay inhibit mutagenesis apparently by blocking metabolic activation by normal (but not by Aroclor 1254-induced) rat-liver microsomal enzymes. The purpose of this communication is to report that a variety of heat-processed meat, bakery and cereal products contain mutagens detectable with the Ames Salmonella/mammalian microsome mutagenicity test.

Prior to mutagenic analysis the foods were subjected to an organic extraction procedure similar to one published previously (Pariza *et al. loc. cit.*). Briefly, the foods were blended in approximately two volumes of 0·1 N-HCl, saturated with sodium chloride (to salt-out protein), filtered through glass wool, and extracted twice with equal volumes of methylene, chloride (designated the *acidic extract*). The aqueous phase was then made basic (pH 10), filtered through glass wool, and extracted three times with methylene chloride (designated the *basic extract*). The methylene chloride extracts (acidic and basic) were passed through anhydrous sodium sulphate, evaporated to dryness under vacuum, redissolved in 20% methanol in chloroform, transferred in predetermined aliquots to test tubes, and evaporated to dryness. The residues were then dissolved in 0·25 ml dimethyl sulphoxide for mutagenesis testing (0·1 ml/plate).

Standard bacterial mutagenesis assays were conducted in duplicate for each concentration tested, by the methods of Ames *et al.* (*Mutation Res.* 1975, **31,** 347) and Drinkwater *et al.* (*J. natn. Cancer Inst.* 1976, **57,** 1323) using *Salmonella typhimurium* TA98 (provided by B. N. Ames). The S-9 mix from the livers of rats pretreated with Aroclor 1254 was utilized at 0·1 ml/assay. For all reported data the revertant colonies obtained were typical, and the light 'background lawn' was normal in appearance indicating that nontoxic doses of the extracts were tested. Tiny (or 'micro') colonies were not observed.

Widely varying levels of mutagenic activity were detected in the heat-processed foods selected for study (Fig. 1). The pattern of the dose-response data indicated that the foods might be classified as having moderately-high levels of mutagenic activity (products 1, 2), low levels of mutagenic activity (products 3–6), and low-marginal levels of mutagenic activity (products 7–10). It is significant that some evidence of mutagenicity was evident in every food tested, a conclusion supported by dose-response data (Fig. 1). Moreover, for the foods with the lowest levels of mutagen content (products 8–10) separate assays conducted on different days confirmed that the detection of mutagenic activity in these foods was reproducible.

We have not yet determined whether the mutagenic activities detected in different foods are due to the same or dissimilar substances, but it is clear from analysis of the basic *v.* acidic extracts that the mutagen(s) present in product no. 7 were not found at detectable levels in products 3–6 or 8–10 (see legend to Fig. 1). However, similar low levels of mutagenic activity were detected in the acidic extracts of the meat products (chicken and beef broth; not shown), whereas mutagenic activity could not be convincingly demonstrated in the acidic extracts of the other seven foods tested (not shown). Interestingly, we have not detected mutagens in the acidic extracts from fried hamburgers although following certain frying conditions (temperature *v.* time) mutagenic activity in the basic extracts is readily detected (Pariza *et al. loc. cit.*). Since mutagenic activity is not present in uncooked hamburger (Commoner *et al. loc. cit.*; Pariza *et al. loc. cit.*) we assume for the present that mutagenicity reported in this communication is the result of cooking, but Sugimura (*Mutation Res.* 1978, **55,** 149) has commented that some naturally-occurring flavenoids are also mutagenic in the Ames test.

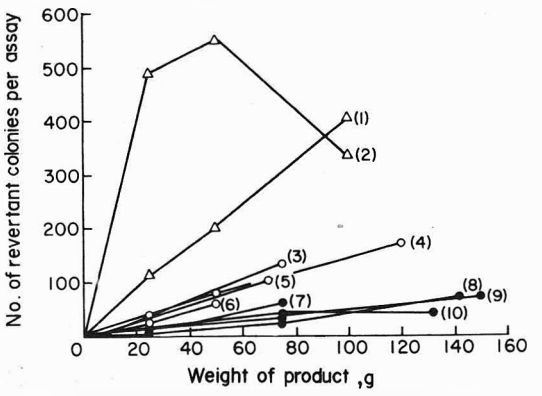

Fig. 1. Mutagenic activity in heat-processed foods. Selected foods were subjected to organic extraction and the results of testing the basic extracts (products 1–6, 8–10) or the acidic extract (product 7) for mutagenicity in the Ames test were plotted against the amount (wet weight) assayed. The symbols represent the means of duplicate assays with background revertant colonies subtracted (solvent controls ± S-9 were 27 colonies per plate or less). Products: (1) canned chicken broth; (2) canned beef broth; (3) crackers; (4) corn flakes; (5) rice cereal; (6) bread crust (soft); (7) Worcestershire sauce; (8) bread crumbs; (9) toast (surface); (10) coconut cookies. The symbols correspond to arbitrary designation as moderately high (>4 revertant colonies/g; △), low (about one revertant colony/g; ○), and low-marginal (about 0·5 revertant colonies/g; ●) levels of mutagenic activity.

The presence of mutagenic activity in cooked foods has been viewed with alarm by some investigators (Commoner *et al. loc. cit.*). In contrast others (Sugimura, *loc. cit.*), including ourselves (Pariza *et al. loc. cit.*), have taken a more cautious approach to the possibility of 'over-interpreting' positive mutagenesis tests using bacterial systems. For example, in this communication we report that a variety of heat-processed foods contained mutagenic activity ranging from low to moderately high. The possible public health significance of these findings, if any, is simply not known at this time. As pointed out by Sugimura (*loc. cit.*), the quantity of mutagenic substances taken in with daily food is likely to be so small that metabolic inactivation processes will be sufficient for adequate detoxification. Furthermore, we have reported that hamburger contains mutagenic inhibitors detectable under some conditions of assay (Pariza *et al. loc. cit.*), and it has also been found (Kada *et al. Mutation Res.* 1978, **53,** 351; Morita *et al. Agric. biol. Chem.* 1978, **42,** 1235) that proteins from certain edible plants inactivate mutagens formed during pyrolysis of amino acids. It is probable that other detoxifying mechanisms also exist within intestinal micro-organisms, etc.

The formation of mutagens in cooked food is not a recent phenomenon, but rather one which has existed throughout the evolution of modern man. Careful and thorough research will be required to elucidate what it means, in terms of public health, to find that virtually all heat-processed foods probably contain mutagens. It is also likely that conditions of temperature and time can be found to reduce the formation of mutagens during heat-processing without sacrificing food quality.

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MEETING ANNOUNCEMENT

CONFERENCE ON XENOBIOCHEMISTRY

The Czechoslovak Medical Society and the Comenius University in Bratislava are to hold an international conference on the "Biochemistry of Metabolism and Effect of Xenobiotics" in Bratislava on 9–13 June 1980. The scientific programme will consist of selected major lectures, poster sessions, and discussion sessions during which up-to-date experimental results will be presented in short communications. The main aspects to be considered will be the control of the metabolism of xenobiotics, their bioactivation and interaction with biological systems, xenobiotic detoxication mechanisms, and applications of mono-oxygenase systems. English will be the official language of the conference.

The Chairman of the Organizing Committee is Prof. A. Jindra, DrSc, and requests for further information should be addressed to The Biochemical Institute, Comenius University, Kalinciakova 8, 880 34 Bratislava, Czechoslovakia.

Fd Cosmet. Toxicol. Vol. 17, p. 433. Pergamon Press 1979. Printed in Great Britain

FORTHCOMING PAPERS

It is hoped to publish the following research papers and other communications in the next issue of *Food and Cosmetics Toxicology*:

Uptake, distribution and metabolism of [^{14}C]amaranth in the female rat. By J. A. Ruddick, J. Craig, B. Stavric, R. F. Willes and B. Collins.

2,6-Di-*tert*-butyl-4-methylene-2,5-cyclohexadienone: a hepatic metabolite of butylated hydroxytoluene in rats. By O. Takahashi and K. Hiraga.

Consumption of toxic rice oil by 'Yusho' patients and its relation to the clinical response and latent period. By H. Hayabuchi, T. Yoshimura and M. Kuratsune.

Lindane induction of microsomal monooxygenases of the rat liver: effects of a high-fat diet. By R. Albrecht, E. Dooh-Priso, F. Faudemay, M. A. Pélissier and J. P. Carreau.

Nitrite sources and nitrosamine formation *in vitro* and *in vivo*. By C. L. Walters, F. P. A. Carr, C. S. Dyke, M. J. Saxby, P. L. R. Smith and R. Walker.

Effects of calcium and sodium carrageenans and ι-carrageenan on hamster foetal development. By T. F. X. Collins, T. N. Black and J. H. Prew.

Effect of graded doses of γ-irradiation on aflatoxin production by *Aspergillus parasiticus* in wheat. By E. Priyadarshini and P. G. Tulpule.

Conversion of aflatoxical to aflatoxin B_1 in rats *in vivo* and in primary hepatocyte culture. By Z. A. Wong, G. M. Decad, J. L. Byard and D. P. H. Hsieh.

Exposure to *N*-nitrosodimethylamine in a leather tannery. By D. P. Rounbehler, I. S. Krull, E. U. Goff, K. M. Mills, J. Morrison, G. S. Edwards, D. H. Fine, J. M. Fajen, G. A. Carson and V. Rheinhold.

Carcinogenicity of the hair-dye component 2-nitro-*p*-phenylenediamine: Induction of eosinophilic hepatocellular neoplasms in female B6C3F1 mice. By G. Reznik and J. M. Ward.

HYDROGEN ENERGY SYSTEM

Proceedings of the Second World Hydrogen Energy Conference, Zurich, Switzerland, August 1978
Edited by: **T.N. VEZIROGLU,** *University of Miami, Florida, USA,*
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SOME PAPERS PRESENTED AT THE CONFERENCE

R. SCHULTEN *(W. Germany).* Nuclear energy as a primary energy source for Hydrogen production

J.H. KELLEY, R. HAGLER Jr. *(USA).* Storage, transmission and distribution of Hydrogen

M.E. NELSON *et al. (USA).* Hydrogen production from nuclear fission product waste heat and use in gas turbines

L.D. RYAN *(USA).* The theoretical design of a solar engine for the production of Hydrogen

A.C.C. TSEUNG *et al. (UK).* Oxygen evolution of Teflon bonded semi-conducting oxide electrodes

J.M. GRAS, J.J. LE COZ *(France).* Asbestos corrosion study in hot caustic potash solution. Silicate ions influence on electrode overvoltages

K.E. COX *(USA).* Irreversibility analysis of Hydrogen separation schemes in thermochemical cycles.

J.H. NORMAN *et al. (USA).* Chemical studies on the general atomic sulfur-iodine thermochemical water-splitting cycle

S.L. SOO *et al (USA).* A steam process for producing Hydrogen from coal

L.D. HADDEN *(USA).* The economics of producing hydrogen from a small air blown coal gasifier

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J.A. FILLO *et al. (USA).* Hydrogen production from fusion reactors coupled with high temperature electrolysis

J.B. PANGBORN *et al. (USA).* Gas distribution equipment in hydrogen service, preliminary findings

E. ANDERSON *et al. (Switzerland).* Analysis of the potential transmission of hydrogen by pipeline in Switzerland

J.J. THIBAULT *(France).* The cryogenic storage of hydrogen

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