ied from asthma and skin
er, Ann. Allergy 1970,
(Withers & Hale, ibid
ust be treated with
y and it is difficult
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ncians (Huang & Bayless,
estinal cramp and diarrhoea
t hypersensitivity to milk,

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e, in which there is im-
. med. Bull. 1967, 23,
hoea result from defective
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299. 26) have reported an
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sive fat, vitamin
m. Blake et al. (Acta
t the gliadin fraction of
ith coeliac disease, and
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n coeliac disease and
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r normal contemporaries.
r food proteins, especially
selected patients, Pock-
65) suggest that, because
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and hence to the develop-
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basis for a gluten in-
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ption syndrome in children

bohydrate-protein complexes,

but antibodies to various starches have been found in normal persons and, in large amounts, in patients whose condition improved after the removal of the relevant starch from their diet (Lietze, J. Asthma Res. 1969, 7, 25). Patients sensitive to penicillin may react to penicillin residues present in milk (Wicher et al. J. Am. med. Ass. 1969, 208, 143) and cases of allergic reactions to tartrazine and other food dyes have been reported (BIBRA Bull. 1971, 10, 174).

In the last two decades, much has been learnt about food intolerance due to a hypersensitivity to food allergens or to enzyme deficiency. The picture is sometimes confused because of the similarities in symptoms or the possible coexistence of the two syndromes. Such difficulties should not deter consideration of what is undoubtedly an important problem. Individual effects may vary from an acute attack of asthma in a young child to mild indigestion in an adult, and the offending food may be difficult to trace or, less frequently, may be as obvious as Küstner's sensitivity to fish. Many children recognize the food they are allergic to better than do their mothers. Mary Jane's tantrums and refusal to eat her dinner of "lovely rice pudding" may well have been due to an instinct for self-preservation rather than to mere temper.

ARTICLES OF GENERAL INTEREST

IF ALL THE SEA WERE PCB...

The polychlorinated biphenyls (PCBs) have now well and truly lined up with DDT and mercury in the ranks of materials recognized as important environmental contaminants. The qualifications for membership of this group are widespread occurrence, relatively high toxicity and, most importantly, non-biodegradability. The PCBs score well in all these categories, a fact borne out by the large number of review articles written on the subject of PCB pollution during the past year or so (Edwards, Chemy Ind. 1971, p.1340; Peakall & Lincer, BioScience 1970, 20, 958; Hammond, Science, N.Y. 1972, 175, 155; Tinker, New Scient. & Sci. J. 1971, 50, 16).

In simple terms, the problem seems to be that the PCBs, used inter alia as plasticizers and solvents in paints, sealants, adhesives and printing inks, are escaping into the environment, probably from sewage combined with industrial effluent, and are slowly accumulating in rivers and sea areas. The structure of the biological food-chains then ensures that these PCBs are widely distributed throughout the animal kingdom, including man. This much is fact (BIBRA Bull. 1971, 10, 8). The exact toxicological significance of PCB residues is not fully understood, however, although any marked accumulation of even a metabolically inert compound must be considered cause for concern. This concern was somewhat alleviated last year by the decision of the Monsanto group, sole producers

From Information Bulletin, British Industrial Biological Research Association, Vol II, No 1 September, 1972

of PCBs in the USA and the UK, to restrict PCB sales to those destined for use in enclosed applications, such as capacitors and transformers, providing a minimal possibility of leakage into the environment (ibid 1971, 10, 11). As PCBs have been known to cause poisoning in man (ibid 1970, 11, 53; Kuratsume et al. RSPHA 81th Repn 1971, 86, 1083) and as they are clearly accumulating in the environment from some of the less controllable sources, this decision represents an important advance in reducing the potential hazard proffered by these compounds.

Levels of PCB residues

Aquatic animals and the birds feeding on them are the obvious material to use as a starting point for a survey of PCB contamination. Furthermore, as the PCBs, especially the more highly chlorinated ones, have an extremely low solubility in water, one would expect them to be principally associated with the sediment on the bottom of seas and to enter into food chains via bottom-living organisms.

Practical verification of this theory has come from Escambia Bay, Florida, where PCB residues of up to 120 and 1·5 ppm have been found in shrimps and crabs respectively (Nimmo et al. Nature, Lond. 1971, 231, 50). The PCBs, which were mainly located in the sediments of the bay, originated from an up-stream chemical plant. Samples taken from places up-stream from the plant were unpolluted. Experimental work, involving exposure of shrimps and crabs to polluted sediment in the laboratory for 30 days, confirmed that both species were capable of absorbing PCBs from their environment to such an extent that the final residue level in the animals after 30 days often exceeded that in the sediment itself. A similar conclusion was reached by William & Zitko (mar. biol. 1971, 9, 213), using the small shrimp, Gammarus oceanicus. In this case, exposure was for shorter periods measured in hours rather than days. The uptake of PCBs was found to depend on both the external PCB concentration and the total exposure period, but after 4 hours there was a reduction in the rate of uptake. In another study (Sanders & Chandler, Bull. env. contam. & Toxicol. (U.S.) 1972, 7, 257), eight invertebrate species, including shrimps, crayfish and several types of immature aquatic insect, were exposed to PCB at concentrations below 3 ppb[a], a level similar to that found in the waters of Escambia Bay. After exposure for 4 days, total body concentrations ranged from 0·2 ppm in crayfish to 16 ppm in the glass shrimp and 52 ppm in daphnia. In scud (Gammarus pseudolimnaeus) exposed to 1·6 ppm PCB, the concentration in the organism reached equilibrium in 14 days and was then 27,500 times higher than the concentration in the water.

As one might expect, the levels found in fish are somewhat less, most commonly in the 0-1 ppm range. In a Canadian survey of fish taken from lakes, rivers and the sea and reported by Zitko (ibid 1971, 6, 464), values of 0·3-0·4 ppm were most common but levels up to 1·01 ppm were recorded for some samples. Much higher residue levels were seen in fish kept experimentally in water containing known concentrations of PCBs (Hansen et al. ibid 1971, 6, 113). For example, a concentration of 5 ppb resulted in levels of over 100 ppm in both pinfish and spot; furthermore this concentration was fatal to some 50-60% of the fish. A level of 1 ppb PCB was tolerated, however, even though residue levels of around 30 ppm were recorded after 30 days and the livers of these animals contained up

[a] b = 10^9 throughout this article.

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to 200 ppm PCB. Interestingly, after 64 days in non-polluted water, the PCB levels of the whole fish fell by some 75%.

Similar figures have been reported in a review of PCB contamination by Eitko & Choi (Fisheries Research Board of Canada Technical Report no.272, 1971). Once again the levels in fish were generally less than 1 ppm, although occasionally figures as high as 10-20 ppm were recorded from waters near highly industrial areas. In aquatic birds, and especially their eggs, higher levels were found, figures of 1-10 ppm being not uncommon. Fish-eating birds seem to have particularly high PCB levels, occasionally in excess of 100 ppm. Other animals have also been shown to accumulate PCBs. The blubber of grey seals, for instance, commonly contains 10-50 ppm PCB, and one sample was found to have a level as high as 1800 ppm. Of greater importance to man, however, is the finding of up to 5 ppm PCBs in some commercial fish oils and of 0·18, 0·21 and 0·47 ppm in margarines manufactured, respectively, from 100% vegetable oils and 60 and 80% marine oils.

In view of the known environmental contamination, it is not surprising that PCBs are to be found in man. Two samples of adipose tissue analysed by Biros et al. (Bull. env. contam. & Toxicol. (U.S.) 1970, 5, 317) yielded 200 and 600 ppm PCB and further work has detected PCBs at a concentration of 0·105 µg/ml (or 3·5 ppm based on the milk fat) in human milk. In the latter case, fatty tissue was also examined and found to contain 5·7 ppm PCB (Acker & Schulte, Naturwissenschaften 1970, 57, 497). However, a survey of 79 lactating women, 32 from villages in New Guinea and 47 from cities in Texas, failed to confirm these findings (Dyment et al. Bull. env. contam. & Toxicol. (U.S.) 1971, 6, 532). While residues of chlorinated hydrocarbon pesticides were present, there was no evidence of PCBs in the milk of any of these women.

Toxicity of PCBs

The results of Hansen et al. (loc.cit.) suggest that the threshold of toxicity of the PCBs to fish lies around 1 ppb. The pink shrimp, Penaeus duorarum, seems equally sensitive (Nimmo et al. Mar. Biol. 1971, 11, 191). The adults were killed within 17-53 days by Aroclor 1254 concentrations of from 2·4-4·3 ppb, but the juvenile shrimps were found to be less resistant and failed to tolerate even 1 ppb. There were indications that a similar increase in susceptibility to PCBs occurred during moulting. Accumulation (up to 510 ppm) occurred in the hepatopancreas of the animals but subsequently the material was transferred to other tissues and eventually lost. There was very little indication of how or why the animals died, but in vitro inhibition of fish ATPase by PCBs was recently reported (Yap et al. Nature, Lond. 1971, 233, 61; Desaiah et al. Biochem. Pharmac. 1972, 21, 857) and the possibility that this may also occur in vivo perhaps deserves further study.

The signs of PCB poisoning are more apparent in birds. For example, chicks fed a diet containing known quantities of PCB have presented with a characteristic group of reactions. Levels of 100-150 ppm PCB (Aroclor 1248) in the diet for up to 5 wk have been shown to depress weight gain, cause oedema, breathlessness, hydropericardium and internal haemorrhages, depress secondary sexual characteristics and increase liver weight (Mehfeld et al. Poult. Sci. 1971, 50, 1090). At 50 ppm these effects were less marked and at 10 ppm they were absent. Month-old cockerels fed a diet containing 500 ppm Aroclor 1254 for up to 12 weeks showed a significant decrease in the size of combs and testicles, a reduction in

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body weight, an increase in liver weight and an accumulation of PCB principally in adipose tissue, kidney, liver, brain, muscle and blood (Platonow & Funnell, Can. J. comp. Med. 1972, 36, 89). Tissue levels increased linearly with time. Behavioural effects have been seen in caged robins receiving as little as 5 µg PCB (Clophen A50) daily, however. After 20 or so days of treatment, the experimental birds were significantly more active than the controls and residue levels in breast muscle (0·1-0·4 ppm) were four times higher (Ulfstrand et al. Nature, Lond. 1971, 231, 467).

Other effects of PCBs in birds include an increase in susceptibility to viral hepatitis seen in ducks challenged with the virus 5 days after the termination of a 10-day course of feeding with diets containing 25-100 ppm Aroclor 1254 (Friend & Trainer, Science, N.Y. 1970, 170, 1314). In pigeons and quails fed wheat dressed with 250-500 ppm Aroclor 1242 or 1254 for 20-28 days, residue levels have been found varying from over 100 ppm in fat to less than 10 ppm in heart and brain (Bailey & Bunyan, Nature, Lond. 1972, 236, 34). The most important result of this work, however, was the finding that PCBs with a relatively low degree of chlorination are fairly rapidly metabolised and are not as persistent as the more highly chlorinated compounds. The anomaly therefore arises that the less widely used compounds, the highly chlorinated PCBs, are more common in the environment than their technologically more popular relations.

The metabolism of an orally administered PCB with a fairly high mean chlorine content (Aroclor 1254) has been studied in the rat by Grant et al. (Bull. env. contam. & Toxicol. (U.S.) 1971, 6, 102). The PCB used consisted of six components recognizable by gas chromatography. In line with the results of Bailey & Bunyan (loc.cit.), tissue residues of the less chlorinated components were significantly lower than the residues of the more chlorinated ones. In addition, total residue figures were increased by treatment of the rats with carbon tetrachloride, a result which, when considered alongside the increase in liver weight observed in this and several other studies, suggests that the liver may be the site of PCB metabolism.

An increase in the activity of the microsomal processing enzymes of the liver has been observed in PCB-treated birds (Bailey & Bunyan, loc.cit.), rabbits (Villeneuve et al. Bull. env. contam. & Toxicol. (U.S.) 1971, 6, 120) and rats (Bickers et al. Res. Commun. chem. Path. Pharmac. 1972, 3, 505). The more highly chlorinated biphenyls were the most potent inducers, producing a statistically significant and long-lasting effect in both species. Reduction of pentobarbitone sleeping time in rats by a number of PCBs has been shown to increase with the degree of chlorination of the compound and the extent of its storage in fatty tissues (Villeneuve et al. Bull. env. contam. & Toxicol. 1972, 7, 264).

In the rabbit study (Villeneuve et al. 1971, loc.cit.) pregnant animals were used, so that additional data were generated showing that 10 mg PCB/kg/day was without effect on this mammalian reproductive system. However, daily doses of 12·5 mg Aroclor 1254/kg or more given to rabbits on days 1-28 of gestation led to an increase in abortions, maternal deaths and stillbirths, although the dead foetuses showed no consistent skeletal abnormalities. The rat appeared to be less sensitive, since doses up to 100 mg/kg/day caused no foetal deaths or malformations (Villeneuve et al. Envir. Physiol. 1971, 1, 67). Placental transfer of the PCBs was verified by human experience during the PCB poisoning incident in Japan (Kuratsune et al. loc.cit.). Pregnant women suffering from the poisoning gave birth to generally small babies with a character-

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istic brown discoloration of the skin, especially around the groin, dark-coloured nails and discharging eyes. The condition disappeared within a few months.

An increase in the metabolic activity of the liver is associated with an increase in smooth endoplasmic reticulum (SER). Proliferation of SER and hyaline degeneration have been observed in the livers of PCB-treated mice and monkeys (Nishizumi, Archs envir. Hlth 1970, 21, 620). Associated with these effects were disturbances in lipid metabolism and a reduction in liver-glycogen stores.

Similar results have been reported in rabbits following the percutaneous absorption of several PCB preparations (Vos & Beems, Toxic. appl. Pharmac. 1971, 19, 617). Besides liver hypertrophy, damage to the kidney tubules, lymphopenia and atrophy of the thymus were noted. The quantities of PCBs involved, 118 mg applied five times weekly for 38 days, also had a marked effect on the skin. The lesions induced were typified by hyperplasia and hyperkeratosis of the epidermal and follicular epithelia. However, as some PCB preparations produced more severe symptoms than others, the possibility that these effects may have been caused by toxic impurities such as benzofurans must be borne in mind.

The sources and the hazard to man

Monsanto's recent refusal to sell PCBs for "open" applications and for use in food-processing plants will clearly, in the long term, reduce the potential hazard of these compounds. At present, however, PCBs are present in human food. The levels involved are low—a recent US estimate put the average daily intake of a person on a high-consumption diet at only 0·1 µg/kg/day (BIBRA Bull. 1972, 11, 169); nevertheless the biological characteristics of the PCBs warrant the continued monitoring of man's exposure to them.

Fish remains man's prime source of PCBs (ibid 1972, 11, 53). Indeed, it is difficult to see how this situation can change in the foreseeable future because, once polluted, the oceans must take a considerable time to recover. Other sources of PCBs can more easily be reduced and eventually eradicated. The finding of PCB residues in poultry and eggs (ibid 1971, 10, 168 & 364) should gradually diminish as PCB-containing meal is removed from the market and the use of biphenyls in food plants is discontinued. Similarly, careful control over the use of PCBs as silo sealants (Skrentny et al. Bull. env. contam. & Toxicol. (U.S.) 1971, 6, 409) should reduce the possibility of residues in milk. PCBs have been found in the milk of cows fed contaminated silage (BIBRA Bull. 1971, 10, 312), and Saschenbrecker (Vet. Rec. 1972, 90, 100) has shown that milk is by far the most important route of excretion of ingested PCB in the lactating cow. After cows have been removed from any PCB contamination, the rate of reduction in the residue level in milk has been found to be similar to that of DDT in the same circumstances (Fries et al. Bull. env. contam. & Toxicol. (U.S.) 1972, 7, 252).

The other important source of dietary PCBs has been food-packaging materials manufactured from recycled papers containing carbonless copy paper (Masuda et al. Nature, Lond. 1972, 237, 41) and printing inks (BIBRA Bull. 1971, 10, 426 & 481). Nevertheless, while PCB levels in packages have occasionally been very high, food itself seems to be only rarely contaminated to any significant extent (ibid 1972, 11, 169). In the UK, manufacturers of carbonless copy paper have stressed that

alternative solvents are now used (Lister & Bennett, Nature, Lond., 1972, 237, 414).

In the USA, the FDA has set a tentative acceptable daily intake for the PCBs of 150-300 µg (BIBRA Bull. 1971, 10, 481). The actual daily intake, even before the recent tolerance levels set for various foodstuffs in America (ibid 1972, 11, 169), was well below this. As PCB residue levels are now likely to fall, it seems that man at least should not suffer unduly from this particular environmental hazard. The effect of PCBs on oceans, lakes and rivers and their flora and fauna may be another question.

TOXICOLOGY'S NEED FOR NEW ANALYTICAL METHODS

At the present time, one of the major problems facing toxicologists is the fact that many of the methods available for analysis of hazardous substances lack either specificity or sensitivity. The purpose of this article is to highlight the problems that arise in establishing chemical identity and measuring small amounts of toxic substances, to indicate some areas in toxicology where improvement may be made, and to suggest some basic approaches that might produce fruitful results.

These topics may be suitably introduced by reference to the comments of a speaker at a symposium held in Boston in April by the American Chemical Society (Food Chemical News 1972, 14 (4), 44). Dr. P. Issenberg of the Massachusetts Institute of Technology is reported as having emphasized the need for new methodological approaches, and particularly for new techniques for the detection of various nitrosamines and their derivatives in a wide variety of foods. He continued by criticising the lack of specific analytical methods and the low instrumental sensitivity that characterised much earlier work in this field, and made a plea for the wider application of mass spectrometry to the problem of nitrosamine detection.

The use of the mass spectrometer coupled to the gas-liquid chromatograph (GLC-MS) is indeed one of the most versatile analytical procedures currently available for the detection of organic compounds, and is widely used for specific determinations of as little as 10^{-15} g (femtogram) quantities of various chemicals. However, the use of this powerful analytical tool is not general in toxicology, except in the measurement of pesticides. For example, in a recent review of the use of mass spectrometry, Millard (Adv. Drug Res. 1971, 6, 157) made virtually no mention of previous uses in connexion with toxicology. Indeed it is sad to note that at a recent international symposium on GLC-MS held in Italy, only two of the 31 contributions were directly concerned with toxicological problems (International Symposium on Gas Chromatography—Mass Spectrometry, Isle of Elba, 17-19 May 1972).

Fields in which GLC-MS is currently generating important data include the study of hallucinogenic drugs, including the measurement of tissue concentrations of the cannabinoids derived from marihuana, and the vast fields of catecholamine research and adenine nucleotide physiology.

From the toxicological viewpoint the field of pesticide determinations shows the most sophisticated methodology. Here the use of the latest techniques has been forced upon the analyst because of the need to detect

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