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Effects of ingesting a combination of 20 or 40 pesticides at ADI levels on carcinogenesis in rats

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Abstract. Modifying effects of pesticide mixtures on tumorigenesis were investigated with our medium-term carcinogenesis protocols. In the 8-week rat liver model, administration of 20 pesticides (19 organophosphorus and one organochlorine), added to the diet each at acceptable daily intake (ADI) levels, did not enhance rat liver preneoplastic lesion development initiated by diethylnitrosamine. In contrast, a mixture of these 20 pesticides at 100 times the ADI significantly increased the number and area of liver lesions. In a multi-organ carcino-genicity protocol of 28 weeks, mixtures of 40 pesticides (high production examples) or 20 pesticides (suspected carcinogens) added to the diet at their respective ADI levels did not modulate carcinogenesis in any organ initiated by 5 carcinogens in combination. These results thus provide direct support for the safety factor approach using ADI values for the quantitative risk valuation of pesticides. Furthermore, these bioassays were particularly useful methods for the safety evaluation of combination toxicities.

1. Introduction

As possible environmental toxic or carcinogenic agents, pesticides deserve particular attention [3,15]. Not only are workers in the industrial and agricultural fields exposed to these chemicals, but the general public is also potentially at risk due to exposure to pesticide residues in foods. Although the assessment of human cancer risk associated with specific chemical exposures is a complicated scientific endeavor, the WHO Expert Group on Pesticide Residues and the Food and Agriculture Organization of the United Nations (FAO), which regularly hold joint meetings on pesticide residues, have set an acceptable daily intake (ADI) for each pesticide as one approach to quantitative risk evaluation [4]. Actually, pesticide residues were found in many samples and some (about 1 % of 3300 chemicals) exceeded maximum residue limits [2].

We have conducted an extensive study of the carcinogenic activity of pesticides in the last few years using our medium-term bioassay system [1,5,9]. In order to confirm the efficacy of this approach using ADIs, and since additive or synergistic

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effects of complex mixtures have become increasingly important for risk estimation in human toxicity [11,14,17,19], we tested the possible carcinogenic influence of mixtures of 20 or 40 pesticides. To do this we used two types of medium-term bioassays for rapid detection of carcinogens; the liver and multi-organ models [11]. All pesticides examined are registered for use in Japan at the present time.

2. Materials and methods

Male F344 rats, 6 weeks old, were used. The ADI values were proposed by the Ministry of Health and Welfare, Japan, according to the JMPR reports [4]. Concentrations of pesticides in the diet (mg/kg diet) were calculated based on our previous food intake and body weight data [10]. Food and water were available *ad libitum*.

Experiment 1: The pesticides investigated, along with concentrations in the diet, their purity and ADIs (mg/kg body weight/day) were: acephate, 0.3 mg/kg (99.3 %, 0.03); butamifos, 0.016 mg/kg (97.9 %, 0.0016); chlorfenvinphos, 0.015 mg/kg (93.3 %, 0.0015); chlorpyrifos, 0.1 mg/kg (99.3 %, 0.01); dichlorvos, 0.033 mg/kg (98.9 %, 0.0033); dimethoate, 0.1 mg/kg (99.0 %, 0.01); edifenphos, 0.025 mg/kg (95.0 %, 0.0025); endosulfan, 0.06 mg/kg (98.0 %, 0.006); etrimfos, 0.03 mg/kg (94.0 %, 0.003); fenitrothion, 0.05 mg/kg (96.7 %, 0.005); iprobenfos, 0.03 mg/kg (94.9 %, 0.003); isoxathion, 0.03 mg/kg (95.2 %, 0.003); malathion, 0.2 mg/kg (95.4 %, 0.02); methidathion, 0.01 mg/kg (92.04 %, 0.001); pirimiphos-methyl, 0.1 mg/kg (99.7 %, 0.01); prothiophos, 0.015 mg/kg (94.7 %, 0.0015); pyraclofos, 0.01 mg/kg (98.4 %, 0.001); tolclofos-methyl, 0.64 mg/kg (99.5 %, 0.064); trichlorfon, 0.1 mg/kg (99.0 %, 0.01); and vamidothion 0.08 mg/kg (99.0 %, 0.008). All pesticides examined are organophosphorus compounds, except for endosulfan.

Fig. 1 shows the experimental protocol for Experiment 1. The animals were initially given a single i.p. injection of diethylnitrosamine (DEN) at a dose of 200 mg/kg. After a 2-week recovery period, the rats received the pesticides either at the ADI level (mixture 1, group 1-a) or at 100 times ADI (group 1-b), or were maintained on the basal diet throughout the experiment (group 2). Groups 3-a and 3-b were injected with saline and then fed on mixture 1 at the ADI and 100 times ADI, respectively. All animals were subjected to two-thirds partial hepatectomy at week 3 and sacrificed at week 8. Liver slices were fixed in ice-cold acetone, embedded in paraffin, and then immunohistochemically stained for glutathione S-transferase placental form (GST-P) – as previously reported [1]. Numbers and areas of GST-P-positive hepatic cell foci larger than 0.2 mm in diameter, and the total area of liver sections examined, were measured using a video image processor.

Experiment 2: Pesticides selected for the mixtures were 40 chemicals of high volume production (mixture 2) and 20 chemicals for which carcinogenicity has been reported or suspected (mixture 3). The pesticides and concentrations (mg/kg diet) were: acephate (0.3), bendicarb (0.04), bensulide (0.4), bentazone (0.9), chinomethionat (0.06), chlorobenzilate (0.2), chlorpropham (1), chlorpyrifos (0.1),

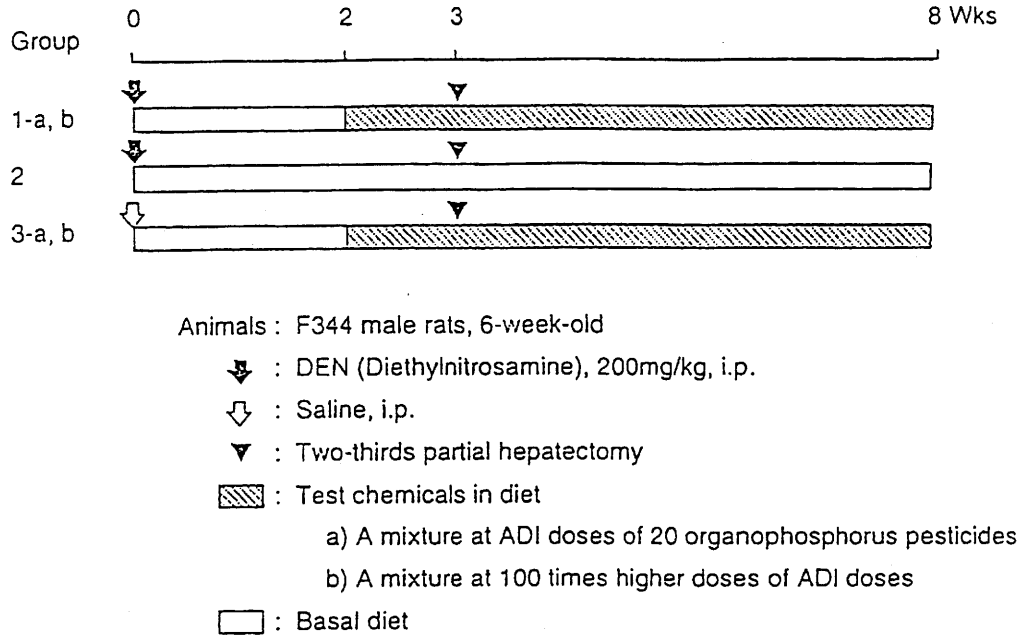

Fig. 1. Experimental protocol of the medium-term liver bioassay (*Experiment 1*)

clofentezine (0.086), cyfluthrin (0.2), cyhalothrin (0.085), cypermethrin (0.5), diflubenzuron (0.12), fenarimol (0.1), fenbutantin oxide (0.3), fenvalerate (0.2), flucythrinate (0.125), flutolanil (0.8), glyphosate (1.5), imazalil (0.25), malathion (0.2), maneb (0.05), mepiquat chloride (0.75), metalaxyl (0.19), metolachlor (0.97), metribuzin (0.125), myclobutanil (0.12), oxamyl (0.2), pendimethalin (0.43), permethrin (0.48), pirimiphos-methyl (0.1), propiconazole (0.18), pyrifenox (1), quinclorac (0.29), methoxydim (1.4), thiobencarb (0.09), triadimefon (0.12), trichlorfon (0.1), vinclozolin (1.215), and zineb (0.05) in mixture 2, and acephate (0.3), amitraz (0.012), captafol (0.5), clofentezine (0.086), cypermethrin (0.5), 2,4-D (3), dichlorvos (0.033), dichlobenil (0.04), dicofol (0.25), fosetly (8.8), glyphosate (1.5), mancozeb (0.5), maneb (0.05), mefolachlor (0.97), permethrin (0.48), phosmet (0.2), propiconazole (0.18), propoxur (0.63), triadimefon (0.12), and trifluralin (0.075) in mixture 3.

Possible modifying effects of these pesticide mixtures on tumorigenesis were investigated using the medium-term multi-organ bioassay (DMBDD model, Fig. 2) [11,6]. At initiation, five known potent carcinogens were given in combination within the first 4 weeks; a single i.p. injection of DEN at a dose of 100 mg/kg body weight at the start of the experiment, i.p. injections of N-methyl-N-nitrosourea (MNU) at a dose of 20 mg/kg body weight on days 2, 5, 8, and 11, and 4 s.c. injections of 1,2-dimethylhydrazine (DMH) at a dose of 40 mg/kg body weight on days 14, 17, 20, and 23, 500 mg/liter N-butyl-N-(4-hydroxybutyl)-nitrosamine (BBN) in the drinking water during weeks 1 and 2, and 1000 mg/liter 2,2'-dihydroxy-di-n-propylnitrosamine (DHPN) in the drinking water during weeks 3 and 4.

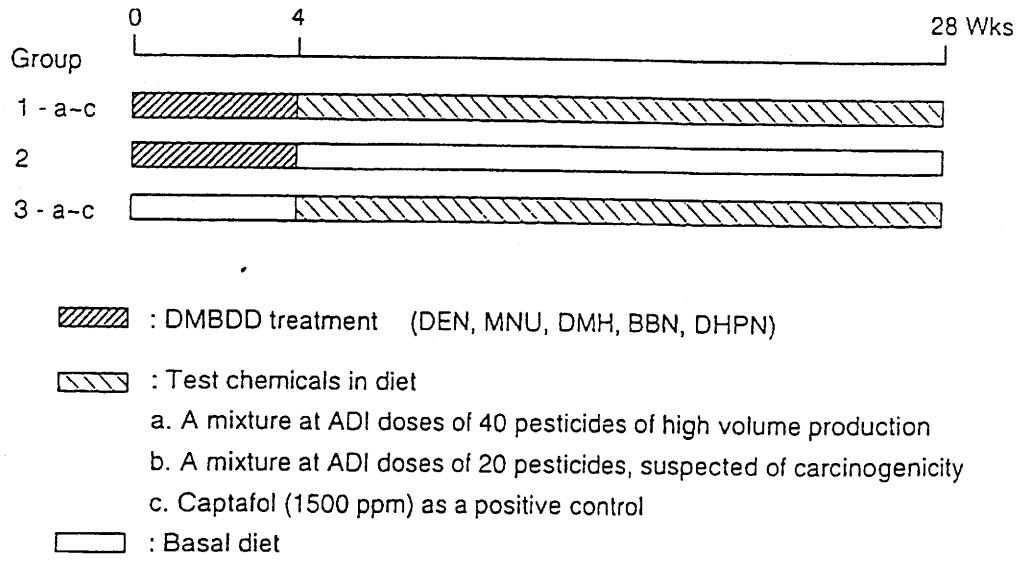

Fig. 2. Experimental protocol of the multi-organ bioassay (*Experiment 2*)

After this DMBDD treatment, groups of rats received one of the pesticide mixtures (group 1 for mixture 2 and group 2 for mixture 3), captafol (1500 mg/kg in the diet) as a positive control (group 3) [16], or the basal diet (group 4) for 24 weeks. Non-initiation controls were injected i.p. with saline and subcutaneously with corn oil and then given pesticide(s) (groups 5–7). At week 28 of the experiment, all surviving animals were killed and completely autopsied. Livers were analyzed as in Experiment 1. The small and large intestines, lungs, and urinary bladders were inflated with 10 % phosphate – buffered formalin, and other main organs and any macroscopic lesions were removed and fixed in formalin. The routinely prepared hematoxylin and eosin sections were examined for neoplastic and preneoplastic lesions.

3. Results

Experiment 1: Based on the food consumption values and average body weights, actual chemical intake was found to be slightly lower than the estimated intake. Body and liver weights were also not influenced by the pesticide administration.

Data on the numbers and areas of GST-P-positive foci per unit area of liver section with and without DEN-initiation are illustrated in Fig. 3. The number of GST-P-positive foci in group 1-a was $3.36 \pm 1.29/cm^2$ and the area of foci was $0.29 \pm 0.15\ mm^2/cm^2$. The levels were essentially the same as those found in the control group ($3.50 \pm 1.29/cm^2$ and $0.28 \pm 0.13\ mm^2/cm^2$). However, the values obtained in the 100 times ADI mixture group (group 1-b) ($4.51 \pm 1.64/cm^2$ and $0.44 \pm 0.20\ mm^2/cm^2$) were both significantly higher than those found in the controls. Without the DEN initiation, neither of the treatment schedules induced GST-P-positive liver cell foci larger than 0.2 mm in diameter (groups 3-a and 3-b).