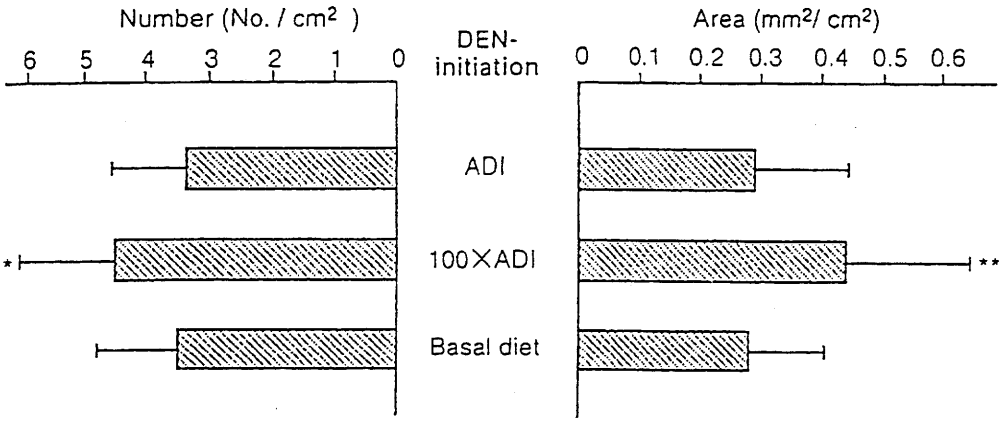

Fig. 3. Numbers and areas of GST-O-positive liever cell foci in *Experiment 1*.
**,* significantly different from basal diet group at $P < 0.01$, $P < 0.05$, respectively.

Experiment 2: No toxic effects were observed for pesticide mixtures in terms of survival rates and weight data. In the liver, development of GST-P-positive foci was increased by captafol but not modulated by the mixtures (Fig. 3). In the other organs, captafol showed promotion effects in the thyroid, whereas the pesticide mixtures did not influence the neoplastic development in any organ (Table 1). No neoplastic and preneoplastic lesions were observed in non-initiated groups (groups 3-ac).

4. Discussion

In the liver model, the ADI mixture of organophosphorus pesticides (mixture 1) exerted no effect on development of liver preneoplastic foci initiated by DEN, although

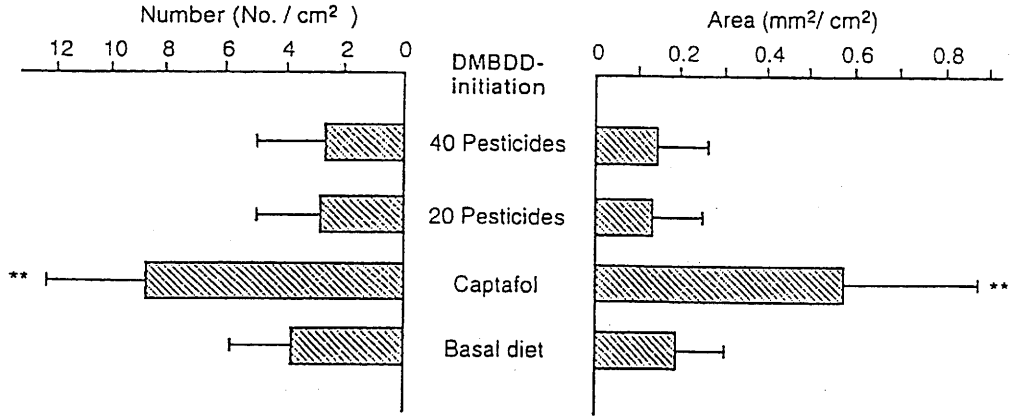

Fig. 4. Numbers and areas of GST-P-positive lever cell foci in *Experiment 2*.
** significantly different from basal diet group at $P < 0.01$.

Table 1. Incidence of tumours (Experiment 2)

Organ and type of tumours	DMBDD-treated group			
	40 pesticides	20 pesticides	Captafol	Basal diet
No. of animals	20	20	19	20
Thyroid				
Follicular adenoma	2	6	9*	2
C-cell adenoma				
Nasal cavity				
Papilloma	1	0	0	0
Odontoma	0	1	0	0
Lung				
Adenoma	4	5	3	5
Carcinoma	1	1	0	2
Oral cavity				
Odontoma	0	2	0	0
Esophagus				
Squamous cell carcinoma	0	0	1	0
Forestomach				
Squamous cell papilloma	3	8	2	4
Squamous cell carcinoma	0	1	1	0
Small intestines				
Adenoma	3	1	2	2
Adenocarcinoma	0	2	2	1
Large intestines				
Leiomyoma	0	1	0	0
Adenocarcinoma	2	2	6	4
Liver				
Hyperplastic nodule	1	0	1	1
Kidney				
Nephroblastoma	2	4	7	2
Transitional cell carcinoma	0	1	0	0
Urinary bladder				
Transitional cell papilloma	0	1	0	1
Prostate				
Leiomyosarcoma	0	1	0	0
Sarcoma, NOS	1	1	0	0
Skin/subcutis				
Squamous cell papilloma	0	1	0	0
Lipoma	0	1	0	0
Abdominal cavity				
Mesothelioma	0	0	1	0
Peripheral nerve				
Malignant schwannoma	0	1	0	0

*: Significantly different from control group at $P < 0.05$.

A few tumors were observed only in the control group: thymic lymphoma (thymus), follicular carcinoma (thyroid), adenocarcinoma (nasal cavity), adenoma (seminal vesicle), keratoacanthoma (skin), schwannoma (peripheral nerve). No neoplastic lesions were found in the non.initiated groups.

the 100 times higher dose demonstrated lesion-promoting potential [10,8,7]. In the multi-organ model, the ADI mixtures of 40 (mixture 2) or 20 (mixture 3) pesticides demonstrated no tumor-promoting potential in any organ or tissue [8,7]. Captafol, on the other hand, exerted apparent tumor- promoting effects in the liver, thyroid and kidney, although the dose level was not comparable to the mixtures. The protocol has been developed in our laboratory over the last 15 years [11]. Quantitative analysis of GST-P-positive foci larger than 0.2 mm in diameter, expressed in terms of number and area per unit area of liver section, has been established. The multi-organ method has been developed to supplement the liver model and also has been demonstrated to be a useful method for rapid detection of carcinogens at a whole body level [11,6].

With a safety factor approach, acceptable exposure levels such as ADIs are usually determined by dividing the no observed effect level (NOEL) from laboratory-based chronic toxicity tests by an appropriately chosen safety factor. The safety factor used for ADI by the Japanese Ministry of Health and Welfare and the FAO/WHO is usually 100, but the WHO expert committees have used figures ranging from 10 to 2000 [13]. Although there are a number of potential problems associated with the safety factor approach, including the fact that the observation of no treatment-related effects may depend on experimental conditions (such as the number of animals exposed and dose levels used), and the fact that biological justification for general use may be lacking [12], the present experimental results indicate that this procedure is indeed appropriate and acceptable for risk evaluation at present. Furthermore, the chance of exposure to so many pesticides (20 or 40 chemicals) in concert might be in practice very low [18].

The observed combination effects at 100 times ADI in Experiment 1, however, suggest that several of the pesticides included in the test are possibly carcinogenic in the liver. Even the mixture of 20 pesticides at ADI levels , for which carcinogenicity has been reported or suspected, exerted no tumor modulating potential in the DMB-DD model.

Since most human cancers may be caused by trace environmental factors, it is of increasing importance that combined effects of chemicals at relatively low doses be examined. The medium-term bioassays used in this document are particularly useful methods for this matter. In conclusion, the present safety factor approach is appropriate for risk evaluation of environmental chemicals.

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