probably undergo oxidation and are not active anymore. It is possible that the polypeptides contained in the hornet venom are responsible for the changes observed in the tadpoles; this will be the subject of further investigation.

Résumé. Le venin de la guêpe (*Vespa orientalis*) supprime la métamorphose du têtard de crapaud (*Bufo viridis*) chez tous les animaux traités au stade prémétamorphique et chez 60% des animaux traités au stade prométamorphique. Onze des animaux traités ont montré un éclaircissement de la peau.

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In vivo Mutagenic Interaction of Nitrite and Ethylenethiourea

Ethylenethiourea (ETU) is by itself a weakly mutagenic[1] and carcinogenic[2,3] decomposition[4] and metabolic[5] product of the fungicidal ethylene-*bis*-dithiocarbamates. Recently it has been reported that ureas and other nitrogen containing pesticides and food contaminants, after nitrosation with sodium nitrite in simulated gastric juice, become highly mutagenic[6-8] and carcinogenic[9,10] substances. Other reports have shown that the feeding of mice or rats with nitrite and amines or amides leads to the in vivo formation of the respective N-nitroso-derivatives[11] and later on in the life of the animals to tumors of various organs[12]. There have been, however, no in vivo mammalian tests for the mutagenic activity of such pesticides interacting with nitrite directly within the animal. For this reason we intended to investigate the effects of an orally given mixture of ETU and sodium nitrite on the cytology of the mouse bone marrow, because the micronucleus test is a simple, rapid, reliable mutagenicity test method[13-15] which is also easy to perform.

We began our experiments with preliminary in vitro studies, in which the *Salmonella typhimurium* tester strains, kindly provided by Prof. B. N. AMES (University of California, Berkeley), were used. The results displayed in Table I show the large increase in the relative mutagenicity when a N-nitrosated ETU was placed onto the test plates.

For our main task, young female ICR mice obtained from the Tierzuchtinstitut of the University of Zürich were given the various test compounds either orally or i.p. Two dosages of each compound were applied. For the i.p. application, the compounds were dissolved in sterile physiological saline, cantaining 3% DMSO. The concentration of each compound was calculated in such a way that a 30 g animal received the proper dose in a volume of 0.50 ml. Orally given compounds were dissolved in a 2% gum Arab solution and were applied with means of a stomach tube. 4 mice were used for each concentration and they received the test substances twice 24 h apart. 6 h after the second application, the mice were sacrificed and the bone marrow smears prepared and stained as described by SCHMID[13].

To differentiate clearly the effects of the ETU-nitrite interaction from those of the single components, we applied them at the same dose levels as used in the mixture. Also included in this test were control animals, which received no treatment, and a positive control with the known mutagen Trenimon (2, 3, 5-*tris*-ethyleneiminobenzoquinone-(1,4).

Chromosome breakage, as an expression of mutational events, leads to the formation of small, separate micronuclei in blood cells. They are easiest to recognize in

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Table I. Relative mutagenic activities of various compounds on *Salmonella typhimurium* strain his G 46

Compound	Concentration (m*M*)	Relative mutagenicity	Comments
None	—	1.0	Spontaneous mutations
ETU	1	2.5	Dissolved in DMSO
$NaNO_2$	5	1.5	Dissolved in acetate buffer (pH 4.5)
ETU + $NaNO_2$	1 + 5	14.0	Dissolved in acetate buffer (pH 4.5)
2-Aminopurine	0.5	10.0	Positive control, dissolved in water

The plate test was performed as outlined by AMES[16] and modified by us[1]. Relative mutagenicity is defined as the quotient from the mean number of colonies on the test plates divided by the mean number of spontaneously arizing colonies.

Table II. Incidence of micronucleated erythrocytes in the bone marrow of mice

Compound	Concentration (mg/kg)	Micronuclei per 1000 polychromatic erythrocytes in Polychromat. erythroc.	Normochromat. erythroc.	Mode of application
None	—	3.8	3.5	—
ETU	450	3.0	2.5	i.p.
	150	2.0	3.5	
$NaNO_2$	150	2.8	2.0	p.o.
	50	2.5	1.3	
N-nitroso-ETU	125	32.0	4.5	i.p.
ETU + $NaNO_2$	600	50.9	2.6	p.o.
(3:1 w/w)	200	28.4	3.1	
Trenimon	0.12	53.5	1.2	i.p.
	0.06	36.7	2.7	
	0.03	18.0	5.0	

erythrocytes, where they are not expelled together with the nucleus. As an added advantage of this test, erythrocytes which are younger than 24 to 30 h stain blueish (polychromatic erythrocytes) instead of red (normochromatic erythrocytes). This allows one to distinguish between micronuclei formed during (and possibly because of) the treatment of the animal and those formed before. By counting micronuclei in poly- and normochromatic erythrocytes separately, the increase of the number of micronuclei due to the treatment is immediately evident. Furthermore false positive results due to viral infections or other conditions resulting in a higher incidence of micronuclei in single animals can thus easily be recognized and excluded.

Our experiments – summarized in Table 2 – showed clearly that the single compounds were mutagenically inactive. Only the single assay with N-nitroso-ETU was seen to increase the number of micronuclei. It was then presumed that the formation of this derivative would also take place in the acid environment of the stomach upon feeding the animals with a mixture of ETU and sodium nitrite. If the so-formed N-nitroso derivative could reach the target cells within the bone marrow, an increase in the number of micronuclei should – and indeed could – be seen. This dramatic increase in the mutagenic activity of 2 substances, when given together, demonstrates again the possibility of a noxious interaction of the food additive sodium nitrite with aminic or amidic food components: Chemical reactions combine in vivo the 2 more or less harmless substances forming a dangerous product, which is then distributed throughout the organism. Having reached a target site, this compound may cause either a mutagenic alteration in a germ cell or malignant transformation of a somatic cell. In the former case, the damage is transmitted to the offspring and to future generations, whereas in the latter case already the exposed individual might develop cancer. The involuntary and indiscriminate uptake of food additives and food contaminants can thus have more or less immediate as well as far reaching effects. The claim that over 80% of the cancer cases are of environmental origin could then well become substantiated by more research in to the areas of chemical interactions. Also more attention has been paid in the last few years to the genetic effects of environmental chemicals. Certainly the time has come to investigate with all possible means the genetic consequences of such combinations and in vivo interactions. Our findings presented here show clearly that the possibility of mutagenic effects through in vivo occurring chemical interaction of two apparently harmless substances has to be taken into account when testing chemicals for safety purposes.

Zusammenfassung. N-Nitroso-Äthylenthioharnstoff induziert Mutationen in *Salmonella typhimurium*. Diese Verbindung wird im Magen von Mäusen aus Natriumnitrit und Äthylenthioharnstoff gebildet; ihre Mutagenität wird mit Hilfe des Mikrokerntests gezeigt.

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A Negative Cooperative Binding Process Between Chloramphenicol and Sodium Dodecyl Sulphate to Bovine Serum Albumin: A Possible Effect on Drug Absorption

Surfactant influence on drug absorption is a well established fact[1]. The mechanism for this effect has been related to different factors, such as drug solubilization above the critical micelle concentration (CMC)[2], wetting of the membrane surface[3–6], structural modifications of the membrane[7,8] and interactions with the dosage form[9,10]. Any influence of premicellar concentrations on membrane transport is generally ascribed to changes in membrane permeability following the interaction of surfactants monomers with one or more biomembrane constituents.

A previous attempt[11] to find a possible explanation to the effect that surfactant monomers might have on