A/J mice were injected with 5×10^6 parasitized erythrocytes per mouse. All 12 mice died 5.5 days after inoculation. Blood smears taken 6 hours before death showed a peripheral parasitemia of 56 to 86 percent. Brain smears stained in Giemsa showed infected cell sequestration and heavy blockage of fine and large brain capillaries in all of these mice.

Past studies with *P. berghei berghei* NK65 and KS11 strains (*9*), in which a systematic search for brain involvement was carried out, failed to reveal any cerebral vascular blockage. In fatal infections of *P. berghei berghei* in hamsters, young albino rats, tree rats, or mice, phagocytosis of hemozoin pigment has been seen in some endothelial cells of brain capillaries. However, the engulfment of malaria pigment in brain capillaries of these animals was minor compared with the hemozoin phagocytosis in the liver, spleen, or lungs. Moreover, no sequestration of infected erythrocytes in brain capillaries has ever been observed in spite of parasitemias of 70 to 85 percent. *Plasmodium vinckei vinckei*, which we have kept for many years in our laboratory, has always produced a fulminating, fatal injection within 6 to 8 days, and terminal parasitemias have ranged from 85 to 100 percent (*10*). In spite of high parasitemias in the lumen of vessels of internal organs and massive hemozoin engulfing, the brains of these mice, when examined in stained smears, crush preparations, or histological sections, were almost free from parasites. Only rarely have infected erythrocytes been seen within a cerebral capillary, and the blood-brain barrier in *P. vinckei vinckei* infected mice has appeared to remain intact until death.

From the results with the virulent *P. b. yoelii 17 x* line, and from our past studies with other rodent malaria parasites, we conclude that the virulence of the *P. b. yoelii 17 x* line is due, at least partially, to its ability to cross the blood-brain barrier, develop intraerythrocytic schizogony within the lumen of cerebral vessels, induce intravascular sequestration of infected erythrocytes, and block small and large capillaries of the brain. We feel this strain is a useful model for the study of physiological and pathological aspects of "cerebral malaria."

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Thiocarbamate Sulfoxides: Potent, Selective, and Biodegradable Herbicides

Abstract. *Sulfoxidation of thiocarbamates yields a new class of chemicals having increased herbicidal activity along with greater tolerance of corn and soybeans in greenhouse tests. However, their thermal stability is not favorable. These sulfoxides are intermediates in the mammalian metabolism of thiocarbamates, being formed by liver microsomal oxidases and cleaved in a system consisting of glutathione and a soluble enzyme from liver.*

The production of agricultural crops is facilitated by the use of effective and selective herbicidal chemicals such as thiocarbamates (Table 1) (*1*). Thiocarbamates undergo rapid biodegradation (*2*), and hence persisting residues are not a problem. However, the mechanism of their biodegradation has not been defined biochemically. We find that thiocarbamate sulfoxides, which form in high yields on treatment of thiocarbamate herbicides with equimolar *m*-chloroperoxybenzoic acid (MCPBA), are not only potent and biodegradable herbicides, but are intermediates in the mammalian metabolism of the thiocarbamates themselves.

Seven commercial thiocarbamate herbicides and many related compounds were treated with equimolar quantities of MCPBA in chloroform or methylene chloride, yielding, in each case, the corresponding thiocarbamate sulfoxide (Table 1). The monooxygenated thiocarbamates are characterized as the sulfoxides by appropriate infrared, nuclear magnetic resonance, and mass spectra and by further reaction with equimolar quantities of MCPBA to give the corresponding sulfones $[RS(O)_2C(O)NR_1R_2]$.

Laboratory tests establish that sulfoxides of EPTC (*S*-ethyl di-*N*,*N*,-propylthiolcarbamate) and pebulate (0.3 to 0.5 part per million) in aqueous solution strongly inhibit the root growth of germinating oat seedlings, whereas concentrations seven to eight times greater are required for the same effect with EPTC and pebulate. Greenhouse and field tests in which the soil and herbicide were mixed before the seeds were planted verify that, in general, the thiocarbamate sulfoxides are more potent herbicides than the corresponding thiocarbamates, particularly when tested on broadleaf weeds and, to a lesser extent, on grass weeds. Table 1 illustrates the increase in potency for control of broadleaf weeds and the reduction in corn injury on sulfoxidation of the thiocarbamates, as judged by greenhouse trials. The crop tolerance is improved by sulfoxidation not only in the case of corn, but also with vernolate on soybeans, although this is not the case with cycloate and pebulate on sugar beets. The selectivity is striking in greenhouse tests on corn and weeds where 0.5 kg/ha (1 kg/ha ~ 1 pound/acre) of the sulfoxides of butylate, EPTC, and vernolate control crabgrass (*Digitaria sanguinalis*), foxtail (*Setaria viridis*), watergrass (*Echinochloa crus-galli*), and wild oat (*Avena fatua*), yet this crop is unharmed even at 27 kg/ha of these sulfoxides. In paddy rice culture, there is little or no advantage in using the sulfoxides of benthiocarb and molinate as compared with the parent thiocarbamates. The sulfoxides have certain advantages over the corresponding thiocarbamates: increased potency in control of some weeds when moist soils are treated; increased safety factor for corn and soybeans, and reduced volatility so that the requirement for immediate incorporation in soil is less critical. Features which may limit their commercial potential are hydro-

Table 1. Potency of seven thiocarbamate [$RSC(O)NR_1R_2$] and thiocarbamate sulfoxide [$RS(O)C(O)NR_1R_2$] herbicide chemicals in control of three broadleaf weeds [curly dock (*Rumex crispus*), redroot pigweed (*Amaranthus retroflexus*), and wild mustard (*Brassica kaber*)] and injury of corn. The herbicide was mixed with the soil before the seeds were planted.

Name of parent thiocarbamate		Substituents			Control of broadleaf weeds at 0.5 kg/ha (av. %)		Injury of corn at 3.4 kg/ha (%)	
Common	Trade	R	R_1	R_2	Thiocarbamate	Thiocarbamate sulfoxide	Thiocarbamate	Thiocarbamate sulfoxide
Benthiocarb	Saturn	4-Cl-$C_6H_4CH_2$	C_2H_5	C_2H_5	0	0	0	0
Butylate	Sutan	C_2H_5	iso-C_4H_9	iso-C_4H_9	23	68	0	0
Cycloate	Ro-Neet	C_2H_5	C_2H_5	Cyclohexyl	23	77	50	0
EPTC	Eptam	C_2H_5	C_3H_7	C_3H_7	58	90	70	0
Molinate	Ordram	C_2H_5	-$CH_2CH_2CH_2CH_2CH_2CH_2$-		17	33	10	0
Pebulate	Tillam	C_3H_7	C_2H_5	C_4H_9	17	63	50	0
Vernolate	Vernam	C_3H_7	C_3H_7	C_3H_7	37	95	80	0

lytic instability in aqueous alkaline medium, an unfavorable degree of stability in storage at ambient and elevated temperatures resulting in the buildup of pressure in closed containers, increased leaching in the soil due to high water solubility, and the necessity for higher levels of soil moisture for effectiveness. The thiocarbamate sulfones are much less effective as herbicides.

EPTC sulfoxide and pebulate sulfoxide are less toxic to mice treated intraperitoneally than are EPTC and pebulate, respectively. Radioactive EPTC and pebulate sulfoxides (labeled with ^{14}C on the α carbon in the *S*-alkyl chain) are rapidly metabolized in mice, in that about 40 percent of the label is liberated as carbon dioxide. In fact, these sulfoxides yield essentially the same amount of $^{14}CO_2$ as the parent thiocarbamates comparably labeled. The urinary metabolite pattern, as revealed by thin-layer chromatography (TLC), is similar or the same, regardless of whether the thiocarbamates or the thiocarbamate sulfoxides are administered to mice. The sulfone of EPTC, which is also rapidly metabolized, gives very little $^{14}CO_2$ as compared to that produced from EPTC or its sulfoxide. These findings suggest not only that the thiocarbamate sulfoxides are easily biodegraded but also that they may be intermediates in the mammalian metabolism of thiocarbamate herbicides. Thiocarbamate sulfoxides are easily detected in the liver of mice 20 minutes after intraperitoneal treatment with EPTC, molinate, pebulate, and vernolate at 1 mmole/kg. These metabolites are transient, being barely detectable 60 minutes after treatment and are not detected when benthiocarb, butylate, and cycloate are administered. Incubation of each of the seven thiocarbamates in a system containing mouse liver microsomes and reduced nicotinamide adenine dinucleotide phosphate (NADPH) yielded the corresponding sulfoxide (Table 1), which was detected by TLC and ninhydrin reagent. No sulfones were detected, as was expected, since they are quite hydrolytically unstable relative to their likely rate of formation, if any. Other ester metabolites that probably arose from oxidation at a carbon rather than the sulfur substituent were also detected in vivo and in vitro. For example, *N*-depropyl EPTC is one metabolite of EPTC in the microsomal oxidase system (*3*).

EPTC is also sulfoxidized in corn plants (*4*), as is butylate in soil (*5*), suggesting that sulfoxidation is one of the initial steps in biodegradation of thiocarbamates under many environmental conditions.

The biodegradation of the thiocarbamate sulfoxides is explained by their rapid reaction with glutathione (GSH) in the presence of mouse liver soluble enzyme (or enzymes), cleaving the carbamoyl bond. It appears likely that GSH *S*-transferase (or transferases) catalyzes the attack of GSH at the carbonyl group of each of the thiocarbamate sulfoxides. Enzymatic cleavage of the thiocarbamates requires both the microsome-NADPH system to form the sulfoxides and the soluble-GSH system for sulfoxide cleavage. Thus, sulfoxidation is an important step and is probably often the rate limiting step in biodegradation of thiocarbamate herbicides, as follows (*3*)

$$\underset{\text{Thiocarbamate}}{RSC(O)NR_1R_2} \xrightarrow[\text{NADPH}]{\text{microsomal enz}}$$

$$\underset{\text{Thiocarbamate sulfoxide}}{RS(O)C(O)NR_1R_2} \xrightarrow[\text{GSH}]{\text{soluble enz}} \text{cleavage products}$$

While it is intriguing to speculate that the thiocarbamates undergo sulfoxidation prior to exerting their herbicidal action, this point has not yet been established. The thiocarbamate sulfoxides should be more effective carbamoylating agents and sources of sulfenic acids than the thiocarbamates if these types of reactions or products are involved in the herbicidal activity. In any case, this "activation" hypothesis requires that susceptible and tolerant plants differ in their relative rates of sulfoxide formation and subsequent breakdown or in the sensitivity or importance of the target enzyme or site involved in their herbicidal action.

Thiocarbamate sulfoxides are a new type of chemical with potentially useful biological properties. Their herbicidal activity in the greenhouse is even greater than that of the thiocarbamates, which are among the most important herbicide chemicals. They are biodegradable compounds and are not expected to yield persisting residues.

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