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Susceptibility of Mink to Methemoglobin Formation

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As a result of their position in food chains mink have become increasingly studied with respect to agents that are known to bioaccumulate such as PCBs, organochlorine insecticides amongst others (Crum, et al., 1993). A recent review of mink toxicology indicated that this species had been assessed for their responses to over 30 agents including disinfectants, heavy metals, rat poisons, and organic contaminants and pesticides (Calabrese et al., 1992). Despite the wide range of agents assessed for effects on mink no broad evaluation of the susceptibility of mink to methemoglobin–forming agents has been undertaken. Consequently, the present study was designed to assess the response of mink red blood cells to seven well–recognized methemoglobin–forming agents.

MATERIALS AND METHODS

Sodium nitrite, copper sulfate, α–naphthol and o–dinitrobenzene were purchased from Sigma Chemical Company, St. Louis, MO. Sodium chlorate and p–dinitrobenzene were obtained from Aldrich Chemical Company, Milwaukee, WI. Sodium chlorite was purchased from Matheson Coleman and Bell, Norwood, OH. All other chemicals were obtained from Sigma Chemical Company unless otherwise stated.

Blood was obtained from Dr. Richard Aulerich, Department of Animal Science, Michigan State University. Blood was collected via heart puncture into heparinized tubes and shipped on ice by over night delivery to the University of Massachusetts, Amherst. The blood was washed 3X with cold 0.85% saline, followed by 1X with cold phosphate buffered saline (PBS), pH 7.4 (Bloom et al., 1983). The final pellet of erythrocytes was suspended in PBS.

Concentrations of agents were determined in preliminary dose–response studies, with responses in the range of 5% to 30% methemoglobin as the target for compound comparisons. Up to six concentrations plus a vehicle control were tested for each agent. Sodium chlorite, sodium chlorate, sodium nitrite, and copper sulfate were

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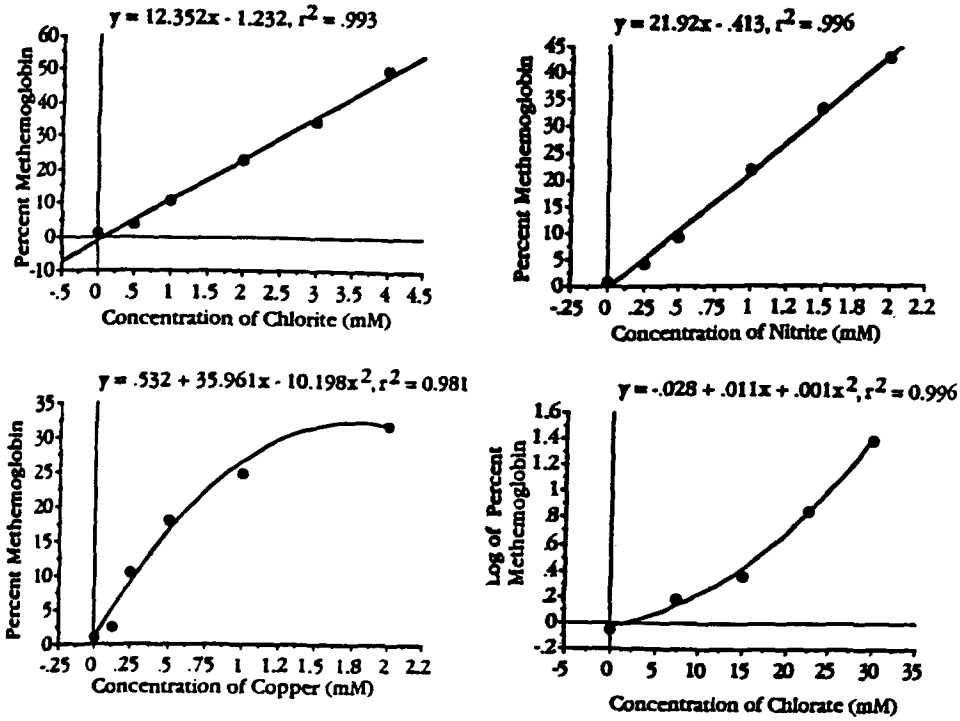

Figure 1. Effect of chlorite, nitrite, copper and chlorate on methemoglobin formation in mink erythrocytes

dissolved in water and added to the erythrocyte suspension. O–dinitrobenzene, p–dinitrobenzene, and α–naphthol were dissolved in acetone. The appropriate aliquot of each chemical was placed into empty test tubes and the acetone was allowed to evaporate in the dark. The erythrocyte suspension was added to the dried tubes. The blood was incubated at 37°C for 1 hour.

After incubation, percent methemoglobin in the erythrocyte suspension was determined according to the method of Brown (1973).

Statistical analyses were performed using Statview II (1987–88, Abacus Concepts, INC., Berkeley, CA). Initially, a one way ANOVA was completed to determine if there was a treatment effect while differences between groups was determined using Scheffe's F–test. Regression analysis was performed to further determine the effect of the chemical dose on methemoglobin formation.

RESULTS AND DISCUSSION

The results of the agent–induced methemoglobin formation in mink erythrocytes in vitro are summarized in Table 1. All seven direct–acting agents produced a dose–

Table 1. Effect of direct–acting agents on methemoglobin formation in mink erythrocytes

Agent	N	Dose (mM)	Methemoglobin % ± SD
Nitrite	10	0.0	0.9 ± 0.5
	8	0.25	4.2 ± 1.3
	10	0.50	9.2 ± 3.8
	10	1.0	21.9 ± 6.5*
	10	1.5	33.5 ± 7.9*
	10	2.0	42.9 ± 11.5*
Chlorite	7	0.0	0.9 ± 0.6
	6	0.5	3.8 ± 1.2
	7	1.0	10.5 ± 1.8*
	7	2.0	22.9 ± 2.8*
	7	3.0	34.3 ± 5.5*
	7	4.0	49.9 ± 10.2*
Chlorate	10	0.0	0.9 ± 0.5
	10	7.5	1.5 ± 0.5
	10	15.0	2.3 ± 1.1
	10	22.5	7.1 ± 4.2
	10	30.0	24.9 ± 9.8*
Copper	7	0.0	0.9 ± 0.6
	6	0.125	2.4 ± 1.5
	7	0.250	10.3 ± 4.8
	7	0.500	17.9 ± 8.7*
	7	1.00	24.8 ± 10.8*
	7	2.00	31.9 ± 11.0*

Table 1. Effect of direct–acting agents on methemoglobin formation in mink erythrocytes (Continued)

Agent	N	Dose (mM)	Methemoglobin % ± SD
p–dinitrobenzene	8	0.0	0.8 ± 0.2
	6	0.02	6.3 ± 1.9
	6	0.04	8.7 ± 3.6*
	6	0.1	9.7 ± 2.3*
	6	0.2	11.7 ± 4.3*
	6	0.5	17.5 ± 3.8*
	6	1.0	19.4 ± 4.6*
o–dinitrobenzene	8	0.0	0.8 ± 0.2
	6	0.2	6.1 ± 2.4
	6	0.4	6.7 ± 1.0
	6	1.0	8.9 ± 1.6
	6	2.0	18.0 ± 9.1*
	6	5.0	22.0 ± 7.7*
	6	10.0	13.3 ± 8.4*
α–naphthol	9	0.0	0.8 ± 0.6
	8	0.06	10.0 ± 4.2
	9	0.13	18.2 ± 6.8
	9	0.25	35.5 ± 12.5*
	9	0.5	51.9 ± 16.6*
	8	1.0	79.8 ± 16.2*

*Statistically different from control as determined by ANOVA ($p < 0.05$) and Scheffe F–test.

Table 2A. Potency ranking of agent induced mean methemoglobin formation in mink erythrocytes based on linear regression

	Slope (m)	Relative Ranking of the Slope
1. α–naphthol	77.07	107.04
2. nitrite	21.92	30.44
3. copper	15.38	21.36
4. p–dinitrobenzene	15.16	21.06
5. chlorite	12.35	17.15
6. o–dinitrobenzene	2.03	2.82
7. chlorate	0.72	1

Table 2B. Potency ranking of agent induced mean methemoglobin formation in Dorset sheep based on linear regression (French, 1992)

	Slope (m)	Relative Ranking of the Slope
1. nitrite	15.14	24.03
2. copper	16.33	25.92
3. p–dinitrobenzene	764.42	1213.37
4. chlorite	4.91	7.79
5. o–dinitrobenzene	79.37	125.98
6. chlorate	0.63	1

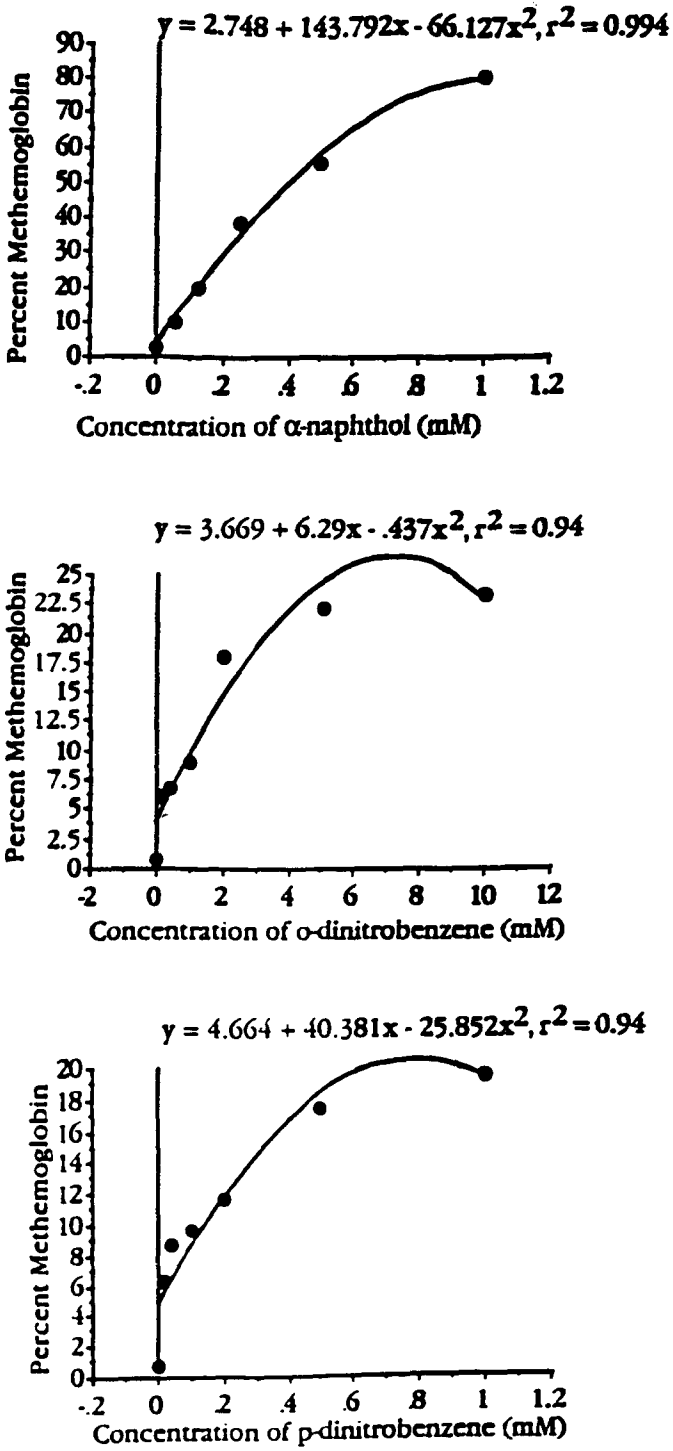

Figure 2. Effect of α–naphthol, o–dinitrobenzene and p–dinitrobenzene on methemo–globin formation in mink erythrocytes

dependent enhancement of methemoglobin formation (Table 1). Results of ANOVA indicated a statistically significant treatment effect for all chemicals.

Both linear and polynomial models were applied to the data and in all cases were able to explain a significant portion of the variability. The model that best fit the data for a particular compound varied – in some cases being linear and in other cases second order polynomial. Figures 1 and 2 show graphs of the results of the regression analysis. A linear model optimally fit the nitrite and chlorite data. A second order polynomial equation more optimally fit a–naphthol, o–dinitrobenzene, p–dinitrobenzene and copper data. The chlorate data was further analyzed by performing a log transformation with a second order polynomial equation providing the best fit for these data.

In order to uniformly compare the potency of the seven compounds, the slopes of the linear dose response relationship was used. Table 2 presents the potency ranking of the agents based on the slope of the linear regression. A relative potency ranking was also determined by dividing the slope of each regression by a reference slope (i.e., the slope of the chlorate regression).

The findings indicate that the mink were responsive to each methemoglobin–forming agent, with nitrite being the most potent and chlorate being the least potent in the present experimental system. The only other direct potency comparison of methemoglobin was recently reported by French et al. (1992) from our laboratory. In that study, six compounds were assessed for their relative ranking of methemoglobin–forming capacity in Dorset sheep using the same study design and concentrations. The sheep displayed striking similarities and differences compared with the mink with respect to some of the slope value relative rankings. For example, in both the sheep and mink, chlorate was the least potent methemoglobin former. The slopes were also similar for both species for nitrite, copper and chlorate. In contrast, p–dinitrobenzene and o–dinitrobenzene were extremely potent methemoglobin formers in sheep but much less so in mink. The mechanisms to account for these interspecies difference remain to be investigated.

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