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Assessment of biochemical and behavioral effects of carbaryl and methomyl in Brown-Norway rats from preweaning to senescence☆

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ABSTRACT

Factors impacting life stage-specific sensitivity to chemicals include toxicokinetic and toxicodynamic changes. To evaluate age-related differences in the biochemical and behavioral impacts of two typical *N*-methyl carbamate pesticides, we systematically compared their dose–response and time-course in preweanling (postnatal day, PND, 18) and adult male Brown Norway rats ($n = 9–10$/dose or time) ranging from adolescence to senescence (1, 4, 12, 24 mo). Carbaryl was administered orally at 3, 7.5, 15, or 22.5 mg/kg and data were collected at 40 min after dosing, or else given at 3 or 15 mg/kg and data collected at 30, 60, 120, and 240 min. Methomyl was studied only in adult and senescent rat (4, 12, 24 mo) in terms of dose–response (0.25. 0.6, 1.25, 2.5 mg/kg) and time-course (1.25 mg/kg at 30, 60, 120, 240 min). Motor activity as well as brain and erythrocyte (RBC) cholinesterase (ChE) activity were measured in the same animals. In the carbaryl dose–response, PND18 rats were the most sensitive to the brain ChE-inhibiting effects of carbaryl, but 12- and 24-mo rats showed more motor activity depression even at similar levels of brain ChE inhibition. We have previously reported that brain ChE inhibition, but not motor activity effects, closely tracked carbaryl tissue levels. There were no age-related differences in methomyl-induced ChE inhibition across doses, but greater motor activity depression was again observed in the 12- and 24-mo rats. Carbaryl time-course data showed that motor activity depression reached a maximum later, and recovered slower, in the 12- and 24-mo rats compared to the younger ages; slowest recovery and maximal effects were seen in the 24-mo rats. Acetylcholinesterase sensitivity (concentration–inhibition curves) was measured *in vitro* using control tissues from each age. Inhibitory concentrations of carbaryl were somewhat lower in PND18, 12-, and 24-mo tissues compared to 1- and 4-mo, but there were no differences with methomyl-treated tissues. Thus, in the dose–response and time-course, there were dissociations between brain ChE inhibition and the magnitude as well as recovery of motor activity changes. The explanation for this dissociation is unclear, and is likely due to early development followed by aging-related decline in both kinetic parameters and neurological responsiveness.

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1. Introduction

Age is considered an important determinant of susceptibility in the general population. Each major life stage (from neonates to aging adults) is characterized by differences in behaviors influencing exposure patterns (*e.g.*,Cohen Hubal et al., 2000; Lawrie, 1998) and in physiology impacting pharmacokinetic parameters (e.g., Clewell et al., 2004; Kinirons and O'Mahony, 2004; Makri et al., 2004; Saghir et al., 2012). Taken together, these factors play a major role in determining age-specific threats to health. The bulk of this literature, however, focuses on the young end of the lifespan, but aging also produces many changes (e.g., Shi and Klotz, 2011; Weinert and Timiras, 2003). Increased susceptibility at any age could be evident as a greater sensitivity to environmental chemicals and/or slower (perhaps incomplete) recovery from acute effects. These differences can be due to toxicokinetic factors that impact tissue levels, and/or toxicodynamic factors that influence the ensuing response. A better understanding of how pesticide exposures are modulated by these age-dependent factors could inform risk decisions for populations representing all life stages.

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N-Methyl carbamate pesticides are widely used in agriculture worldwide, in some cases replacing the more persistent organophosphorous pesticides. There is considerable literature on the neurotoxicity of carbamates, and carbaryl specifically, in a variety of laboratory and environmental species, including humans. Acetylcholinesterase (AChE) inhibition is considered the primary mode of action of carbamates (Ecobichon, 2001) and is the endpoint used in most regulatory decisions (US EPA, 2000). In general, toxicity (cholinergic over-stimulation, altered behaviors, respiratory distress, convulsions) correlates well with the magnitude of AChE inhibition. Carbaryl and methomyl, like the rest of this chemical class, do not require metabolic activation, bind reversibly to AChE, and are rapidly eliminated from the body, thus producing signs of toxicity with a rapid onset and that are relatively short-lived (within hours of exposure; Dorough, 1970). Neurotoxic effects of carbaryl have been measured using a variety of behavioral techniques; for example, motor activity (McDaniel et al., 2007; Ruppert et al., 1983), functional observational battery (Moser, 1995; Moser et al., 1988), operant responding (Anger and Wilson, 1980; Heise and Hudson, 1985), and others. Fewer studies, however, are available describing behavioral effects of methomyl (McDaniel et al., 2007; Moser et al., 2012). Using a within-subject design, we have shown that motor activity is a sensitive indicator of neurotoxicity in adult laboratory rats, showing a linear relationship with cholinesterase (ChE) inhibition following acute exposure to several carbamates, including carbaryl and methomyl (McDaniel et al., 2007).

Despite interest in age-related susceptibility to pesticides (NRC, 1993), there have been few reports in the literature comparing neurotoxic outcomes in very young and adult laboratory animals following exposure to carbamates. Lethality produced by carbaryl was greater in weanling rats (23 days old) compared to adults (Brodeur and DuBois, 1963). We reported that very young rats (11 days old) showed more ChE inhibition following carbaryl compared to older preweaning (17 days) and adult rats (Moser et al., 2010). Although Mack and Gordon, (2007) reported less sensitivity in preweaning rats, the thermoregulatory endpoint was confounded with maternal warming since the pups were nested with their mother. Another study compared behavioral and physiological responses to carbaryl in adult (3 mo) and older (12 mo) rats and reported no age-related differences in effects on tests of motor function, but the older rats showed greater sensory and thermoregulatory changes (Takahashi et al., 1991). At least for carbaryl, these few studies suggest greater sensitivity in the very young as well as the aged compared to adult rats.

For methomyl, similar lethal (LD50) doses were reported for adult and weanling (4–6 weeks old) rats (Gaines and Linder, 1986). A comparative study submitted by the registrant reported that brain ChE in PND11 rats was slightly more sensitive to inhibition than in adult rats (US EPA, 2006). No other studies directly comparing effects of sublethal doses could be found. However, comparing across two studies (McDaniel et al., 2007; Moser et al., 2010), neurological effects (brain and RBC ChE inhibition, motor activity) were not obviously greater in PND17 rats compared to adults. Calculations of ED50 (50% effective dose) values for inhibition of brain ChE showed that pups were slightly more sensitive, with ratio of adult:PND17 values of 1.4 (Moser, 2011). No other studies of age-related differences in methomyl toxicity could be identified.

In order to systematically evaluate potential differences in response across the lifespan, we determined the time-course and dose–response of acute effects of carbaryl and methomyl in rats of different ages. Behavioral effects were quantified using motor activity, following which brains and red blood cells (RBC) were obtained for ChE measurement. The reversible nature of binding to AChE impacts *ex vivo* studies of AChE inhibition, as the dilutions and physiological temperatures used in the more standard assays promote reversal of the carbamate binding to the enzyme (Johnson and Russell, 1975). We therefore used a radiometric assay developed to address these issues, which has been more useful in accurately measuring AChE inhibition following *in vivo* exposure (Winteringham and Fowler, 1966). We also assessed potential inherent differences in sensitivity of the AChE enzyme to carbamate inhibition by conducting *in vitro* assays using control tissues of different ages.

2. Methods

2.1. Animals

Brown-Norway rats (Charles River Laboratories, Raleigh, NC) were housed on hardwood chip bedding (Beta-Chip®) with Enviro-Dri® (enrichment) in an AAALAC International-accredited animal facility with regulated temperature (21 ± 2 °C) and humidity (50% ± 10%). Food (Purina Rodent Chow) and water (filtered tap) were freely available. For carbaryl testing in young rats, time-pregnant females were obtained and allowed to deliver normally. Pups were grouped by sex and redistributed to the dams on postnatal day (PND) 2 or 3, assuring that littermates were spread across litters. Litters were culled to two or three pups (average natural litter size was about three), with as many males as possible. Pups in each litter were dosed in a split-litter design to spread the dose groups across maternal units, *i.e.*, no more than one pup within a litter receiving the same treatment. Rats to be tested at 1 mo were shipped on PND25 and tested on PND32. For both methomyl and carbaryl, young adults (4 mo) or retired breeders (between about 8–12 mo of age) were purchased. In these Brown-Norway rats, there was about 98% survival at 12 mo of age, and 91% survival at 24 mo. Maximum weights were about 500–550 g. A total of 9–10 rats/treatment/age were used for each study. Only male rats were used.

For carbaryl, studies took place at five ages: PND18, and at 1, 4, 12, and 24 mo, whereas for methomyl, an abbreviated experimental design only included 4-, 12-, and 24-mo rats. Two doses were used for the carbaryl time-course to assess potential dose-dependent differences, and only one methomyl dose was used. Each study was conducted independently, but the dose–response and time-course studies for any age less than 12 months were scheduled within a timeframe of several months. Since the retired breeders had a range of birth dates, squads of rats were tested at times, spread over several months, that closely approximated the nominal age (either 12 or 24 mo, within one week).

Table 1
Control values for each measure at each age for each carbamate tested: data from time-course and dose–response studies are combined for each carbamate. Group means and coefficient of variation (standard deviation expressed as a percent of mean) are presented. $N = 26–30$ for each value.

	Brain ChE[a]	RBC ChE[b]	Horizontal counts[c]	Vertical counts[c]
Carbaryl				
PND18	4.98; 6.9%	0.427; 33.0%	83.1; 50.5%	NA
1 mo	6.04; 6.6%	0.342; 41.8%	129.3; 12.0%	NA
4 mo	6.16; 8.0%	0.284; 20.4%	130.8; 14.9%	22.7; 37.4%
12 mo	5.68; 6.5%	0.285; 40.1%	122.4; 20.7%	22.5; 56.1%
24 mo	4.98; 6.5%	0.226; 31.3%	107.5; 28.9%	21.1; 45.3%
Methomyl				
4 mo	6.25; 7.3%	0.256; 15.7%	138.8; 13.5%	27.2; 35.7%
12 mo	5.84; 6.3%	0.290; 27.3%	125.8; 12.7%	23.4; 43.1%
24 mo	6.33; 11.7%	0.311; 26.5%	115.3; 17.2%	19.7; 45.5%

[a] μmol ACh hydrolyzed/g tissue/min.
[b] μmol ACh hydrolyzed/ml RBC/min.
[c] Counts summed over 20-min session.