

# Animal Studies of Methylmercury and PCBs: What Do They Tell Us About Expected Effects in Humans?

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**Abstract:** Methylmercury and polychlorinated biphenyls (PCBs) exemplify the important interactions that should take place between epidemiological and laboratory investigations of developmental neurotoxicants. Often found in the same source, perhaps with multiplicative interactions, it is difficult to isolate specific profiles of effects without advanced behavioral procedures and controlled exposures using laboratory animals. The present review focuses on the effects of developmental exposure to methylmercury or PCBs as expressed in adult animals. The PCBs are subdivided into two structural classes, non-ortho-substituted ("coplanar" or "dioxin-like") PCBs and ortho-substituted ("non-coplanar") PCBs, a distinction supported by different behavioral profiles and neural mechanisms of action. Methylmercury's profile is dominated by sensory effects with a likely cortical site of action. Some of these effects may be amplified with aging. Methylmercury's effects on functions generally termed cognitive can be understood by distinguishing between those reflecting the acquisition of a response-consequence relationship from those reflecting memory or contextual influences over behavior. Methylmercury does not appear to impair memory or discriminations, but retards acquisition of a response-reinforcer relationship. Like methylmercury, non-ortho-substituted PCBs do not appear to degrade memory and contextual control. Ortho-substituted PCBs impair performance on certain spatially-based discrimination and memory tasks. Methylmercury and non-ortho-substituted PCBs disturb the temporal pattern seen in fixed-interval schedules, but apparently without a significant change in the pattern of interresponse times. The ortho-substituted PCBs disrupted this pattern, but did so by increasing the number of short interresponse times. © 2000 Intox Press, Inc.

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## INTRODUCTION

The evolution of our understanding of the risks and hazards associated with a neurotoxicant follows a common pattern. The first evidence usually derives from a disaster, which can be characterized as exposure of a circumscribed population to levels so high that they precipitate overt, ineluctable signs of toxicity. Examples are many and would certainly include the Minamata tragedy (Harada, 1995; Smith and Smith, 1975). Even when the presence of poisoning is clear and derived from a single toxicant, it can be difficult to identify its source.

Such episodes often spark further epidemiological research in which a more broadly based population is

carefully selected, controls are included, and subtle effects are sought in an attempt to link the degree of exposure to the magnitude or prevalence of the effect. Regardless of how carefully the epidemiological study is conducted, however, the results are inevitably correlational so firm conclusions about causation can not be established.

Disasters also motivate studies with laboratory animals where exposure levels and control conditions can be determined precisely by the experimenter, permitting one to tease apart the many complexities inherent in characterizing the actions of neurotoxicants. Animal studies support more solid conclusions about dose-effect relationships and the direction of causality.

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Behavioral studies are a necessary component of any comprehensive assessment of neurotoxicity; alone or in conjunction with other levels of analysis, behavioral effects may contribute to the assessment of risk (Weiss, 1988). Refined behavioral analyses can provide important information about effects on sensory systems (Maurissen, 1995; Rice, 1994), learning (Newland and Reile, 1999b), memory (Miller and Eckerman, 1986), motor function (Newland, 1995; Newland, 1997), and the full expression of complex behavior as revealed by establishing it under certain schedules of reinforcement (Rice, 1988; Weiss and Cory-Slechta, 1994).

Refined behavioral assessments can support a fuller understanding of brain-behavior relationships and how they are affected by neurotoxicant exposure. These assessments can also be used to identify specific effects to be expected from exposure to a particular substance. Effects that are consistently identified in animal studies should be sought in exposed humans. Effects not detected in animal studies should also be absent from exposed humans. Insofar as visual systems are similar, for example, neurotoxicant effects on visual function might be expected to be similar, too. Thus, behavioral assessment of neurotoxicant exposure conducted with animals can guide the planning and interpretation of epidemiological research. This approach is based on an assumption of continuity of effects across species that often needs to be demonstrated. Certainly, it is necessary to examine multiple species before settling on the appropriate model for human exposures, as the Thalidomide episode clearly demonstrated (Newman *et al.*, 1993; Schumacher *et al.*, 1968).

### METHYLMERCURY AND PCBs

These compounds are excellent examples of the progression from acute episodes of exposure to the interaction of epidemiology and laboratory research. Methylmercury and certain polychlorinated biphenyls (PCBs) are well established as neurotoxicants, especially to the developing organism. Methylmercury and the PCBs are often found in the same source, like fish or other aquatic species, thus making it difficult to isolate these compounds' toxicities strictly from epidemiological studies. Successful isolation is important for at least two somewhat related reasons, one pertaining to risk assessment and the other to potential interactions between the two neurotoxicants. First, the assessment of risk associated with, say, methylmercury alone is difficult to determine if populations also are exposed to other hazards. Second, the presence of methylmercury and PCBs in similar sources results in exposure to these compounds simultaneously, and indications that the two may act synergistically (Bemis and Seegal, 1999) increase the importance of understanding their mechanisms of action and profiles of effects.

The present review will examine the functional domains affected by developmental exposure to methylmercury and PCBs. A comprehensive review of both methylmercury or the PCBs would be too large a task for a single paper, and is therefore not attempted here. Instead, the goal is to examine functional domains described in the animal literature to determine the extent to which effects of methylmercury and PCBs overlap or can be differentiated. Sensory and motor endpoints have been well researched with methylmercury and some conclusions can be drawn about its effects. Schedule-controlled behavior has also been examined with these compounds and will be reviewed. Cognitive endpoints will be divided into those that involve the acquisition of a response-reinforcer relationship and those that entail remembering or discriminative control over behavior, a distinction that may help differentiate effects of the classes of neurotoxicants under review.

Effects seen in juveniles or adults that are associated with developmental exposure will be emphasized. This restriction is especially important to methylmercury's neurotoxicity since exposure during adulthood can produce a different profile of effects than developmental exposure. Effects strictly on the course of development (*e.g.*, developmental delays), especially as seen in rodents, will not be covered in detail. This is necessary in part to maintain focus and in part because generality across species in the timing of developmental landmarks is a complex issue requiring separate treatment. Such delays are not excluded completely, and are referred to especially when studied with non-human primates, because of the relative facility in forging comparisons with human development.

Methylmercury is the most common form of mercury exposure. It is lipid soluble, and only weakly soluble in water, so its concentration in an aquatic system can be quite low. However, it is taken up by microorganisms and, as it wends its way through the food web to large predators, its concentration increases to such an extent that levels in long-lived, predatory fish can be 5 orders of magnitude higher than concentrations seen in the water itself (EPA, 1996). Large predators, such as tuna, swordfish, bass, or pike can accumulate high levels of methylmercury, in excess of 1 ppm in their flesh, over the course of a long lifespan (Bahnick *et al.*, 1994; EPA, 1996; Inskip and Piotrowski, 1985).

The PCBs form a class of 209 possible congeners (Silberhorn *et al.*, 1990), making a characterization of this class of compounds an intimidating task. However, an hypothesis has been advanced that permits a classification of these compounds according to a structural distinction (Kodavanti and Tilson, 1997; Seegal, 1995; Shain *et al.*, 1991). Each phenyl ring in a biphenyl can be visualized as forming a separate plane (Figure 1, bottom). With chlorine substitution in the *meta* (m) or *para* (p)

positions, but not in the *ortho* (o) position, the PCB molecule can assume a coplanar formation that is flat, like the tetrachlorodibenzo-*p*-dioxin (TCDD) molecule (McKinney and Waller, 1994). The effects of these non-*ortho*-substituted, "dioxin-like," coplanar compounds are influenced by other structural features, such as the specific pattern of chlorine substitution in the *para* and *meta* positions. The potency of a coplanar PCB relative to dioxin is reflected in a "toxic-equivalency factor" based on its ability to bind the aryl hydrocarbon (Ah) receptor, which appears to mediate the toxic effects of TCDD and related compounds (Safe, 1990; Van den Berg *et al.*, 1998).

Alternatively, the two planes could be oriented at an angle (non-coplanar). This position is associated with poor affinity for the Ah receptor, although it is less clear what non-coplanar PCBs do bind to. The presence of chlorine atoms near the linkage of the two rings, the *ortho* positions, inhibits the formation of a coplanar structure and of Ah binding (Chauhan *et al.*, 2000; Kodavanti and Tilson, 1997; McKinney and Waller, 1994). *Ortho*-substituted PCBs have been linked to PCB accumulation in the nervous system, altered calcium homeostasis and dopamine concentrations in neurons, and a range of

neurotoxic effects (Bemis and Seegal, 1999; Kodavanti and Tilson, 1997; Seegal, 1995; Shain *et al.*, 1991; Tilson and Kodavanti, 1997; Tilson and Kodavanti, 1998). Depending on other structural characteristics, such as *para*- and *meta*- substitution, PCBs can interact with thyroid hormones and disrupt their regulation, actions that could account for some of the developmental neurotoxicity seen with certain PCBs (Chauhan *et al.*, 2000; McKinney and Waller, 1994).

Both non-*ortho*-substituted and *ortho*-substituted PCBs are toxic, but their profiles and mechanisms of toxicity differ. Because of this, the ability of the toxic equivalencies, which are based on TCDD's action at the Ah receptor, to predict the neurotoxicity of *ortho*-substituted PCBs is low (Giesy and Kannan, 1998; Seegal, 1995; Shain *et al.*, 1991). Because of the importance of the structural distinction, separate treatment will be afforded non-*ortho*-substituted (coplanar) and *ortho*-substituted PCB's where possible. In most cases the distinction in structure corresponds to differences in effect, but it should be recognized that the coplanar/non-coplanar *ortho*/non-*ortho* distinction is not a strictly dichotomous classification either in terms of structure or of effect. For the purpose of the present review, however, PCBs will be referred to as "ortho" and "non-ortho" and for the compounds discussed this corresponds to non-coplanar and coplanar, respectively, unless specifically stated otherwise.

Often, PCBs are administered as a mixture. While non-*ortho*-substituted or *ortho*-substituted species may dominate a particular mixture, it is not necessarily the case that the dominant compound in a mixture will also dominate its neurotoxicity because of differences in bioavailability, kinetics, and receptor activity.

## SENSORY EFFECTS

The examination of sensory function in nonhuman species, an area known as animal psychophysics, can be accomplished using reflexive techniques (Crofton, 1990; Young and Fechter, 1983), preferential looking in infants (Gunderson *et al.*, 1986; Gunderson *et al.*, 1988b), respondent conditioning, or operant conditioning (Maurissen, 1988; Maurissen, 1995; Rice, 1994). In the methylmercury literature operant techniques are the dominant, though not exclusive, technique employed. Here, an animal is trained to engage in one response in the presence of one stimulus and a different response in the presence of a second stimulus. The training of such a discrimination is straightforward, behaviorally, because it draws from principles that have long been established and are well-understood. The establishment of a psychophysical profile in an animal is technically demanding, however, due to the many complexities associated with the scheduling of trials, ensuring that the

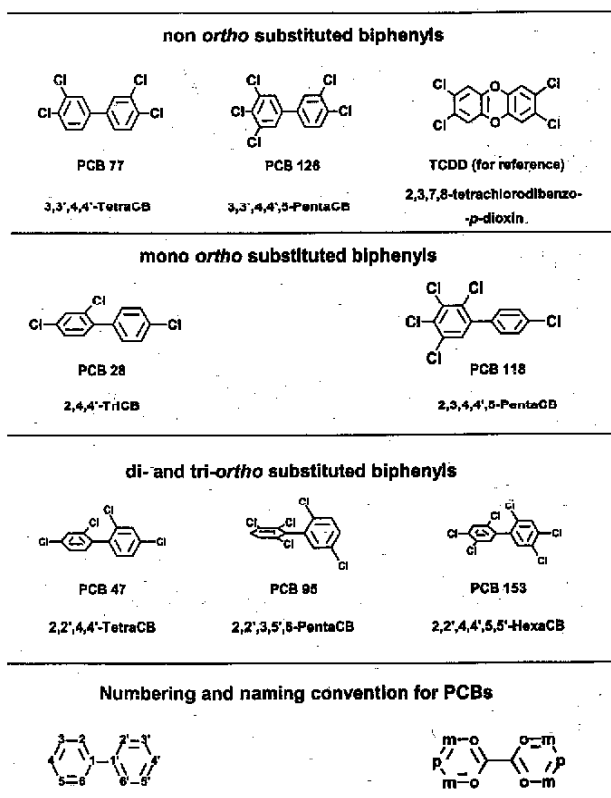


FIG. 1. The IUPAC name, chemical name, and structure of the PCB congeners referred to in the present review. Non-, mono-, and di-/tri-*ortho*-substituted PCBs are shown separately. Non-*ortho*-substituted can assume coplanar and non-coplanar shapes, but only the coplanar shapes are shown here. The mono-, di-, and tri-*ortho*-substituted are rotated to show their preferred non-coplanar conformation. Nomenclature is illustrated at the bottom.

animal is attending, maintaining the discrimination, arranging appropriate reinforcement contingencies, avoiding bias, presenting an appropriate and quantifiable stimulus, and insuring that the discrimination lies along the intended sensory dimension. Few laboratories are capable of accomplishing this.

## Methylmercury

Most of the data on the sensory effects of developmental exposure to methylmercury come from cohorts raised at the Canadian Health Protection Branch (Rice, 1996b). One cohort of macaques was exposed to methylmercury (0, 10, 25, or 50  $\mu\text{g}/\text{kg}/\text{day}$ ) during gestation and through development until approximately puberty, about 3.5-4.5 years old. A second cohort was exposed postnatally to 0 or 50  $\mu\text{g}/\text{kg}/\text{day}$  beginning one day after birth and continuing to 7 years of age.

Because of the investment of resources in these monkeys and the expense of primate research, the monkeys were followed throughout life and tested on a range of procedures, including sensory function. This tactic carries the advantage that much information is acquired from each individual monkey throughout life, and comparisons of performance on different tests can be conducted. Some monkeys were maintained to senescence so age-related changes could be tracked. The disadvantage, of course, is that much of our understanding of mercury's neurotoxicity derives from a single population of subjects. Replication within the laboratory alleviates some concerns, as does the wide range of tests performed, a range that permits the determination of functions that are and, equally important, are not affected.

**Audition.** To test audition, monkeys were trained to touch a stainless steel bar to initiate a trial and release it if a pure tone was presented to one ear during that trial (Rice and Gilbert, 1992). Tones varied in frequency across sessions, and in amplitude within a session. Thresholds describing the minimal amplitude required to report hearing a tone of a particular frequency were taken from all monkeys. Those taken from the control monkeys replicated auditory thresholds typical of macaques; hearing was best in the range of 1000 to about 25,000 Hz, with little variability seen across controls. Low- and mid-range hearing in monkeys exposed postnatally to 7 years old to methylmercury resembled that of controls, but high-frequency hearing was severely impaired. Where thresholds for controls increased between 25,000 and 31,500 Hz, the threshold for exposed monkeys began increasing at 4,000 to 25,000 Hz, representing losses of high-frequency hearing of one full octave or more. The magnitude of the hearing loss at 25,000 Hz was as much as 40-50 dB, a huge loss. This hearing loss, detected seven years after exposure terminated, occurred in the absence

of other behavioral deficits such as discrimination of supra-threshold stimuli, response rate, or reaction time.

In another study, monkeys exposed throughout gestation and postnatally to four years old were tested at 11 and 19 years of age (Rice, 1998a). The hearing loss in these monkeys was seen across a broad range of frequencies. Interestingly, testing conducted at two ages provided some evidence that the age-related declines in hearing began earlier in exposed monkeys.

In another lab, auditory function in methylmercury-exposed rats was examined using reflex modification techniques (Goldey *et al.*, 1994). In this procedure, rats were presented with a white noise stimulus loud enough (120 db for 40 msec) to induce a startle response. This tone was immediately preceded by a pure tone stimulus (intensity ranged 90 db down to threshold), a "prepulse" that inhibits the startle response. To the extent that the startle response is inhibited, it can be concluded that the pure tone forming the prepulse was detected (Crofton, 1990).

On gestational day 6 to 15, rats were exposed to 1, 2, or 4 mg/kg of methylmercury, exposure levels that should be neurotoxic (Burbacher *et al.*, 1990a; Magos, 1987; Magos and Butler, 1976). The highest dose showed reproductive toxicity, but no exposure-related effects on auditory function were detected at any dose when evaluated with reflex modification. Because of the many differences between this investigation and the study with monkeys described above, it is impossible to isolate a single cause for the discrepancy between the two reports, but two differences, in addition to species, seem particularly important: the method of testing and the developmental stage during dosing. As pointed out by Goldey and colleagues (Goldey *et al.*, 1994) reflex modification is best at detecting damage in the peripheral sense organ while operant techniques are also sensitive to deficits in higher levels of the auditory system. In short, the reflex modification might miss methylmercury's effects if the site of damage is cortical, which is why it is important to distinguish between the psychophysical methods used in the two studies. The developmental stage may also have been a crucial difference. Goldey and colleagues (Goldey *et al.*, 1994) used prenatal exposure only while the monkeys reported on by Rice and Gilbert experienced postnatal exposure, some exclusively. When evaluating visual function (described below) this was an important difference; monkeys exposed during postnatal periods were profoundly affected while those exposed during both pre- and postnatal periods were less impaired (Rice and Gilbert, 1992). In a recently published abstract (Burbacher *et al.*, 1999), monkeys exposed only during gestation to methylmercury showed no evidence of hearing loss when tested at 15 years of age.

**Visual Function.** The profile of toxicity in the visual system distinguishes developmental exposure to methylmercury from adult exposure as well as from

exposure to other neurotoxicants (Rice and Gilbert, 1982; Rice and Gilbert, 1990). Visual function was examined in monkeys from the two cohorts, one exposed both during gestation and postnatally and another exposed only postnatally. The latter group showed clear signs of visual damage and the pattern of effects reported suggests a site of damage at a higher level than the retina, and perhaps in the cortex.

In order to understand the effects reported, it is important to recognize that the visual system must assimilate information spread out in space and in time. To organize spatial patterns from the many photons that fall on the retina, the mammalian visual system first

organizes this visual information into abstract spatial patterns. Separate systems exist to handle broad strokes and fine details. In primates, for example, coarse features such as, say, the shape of a zebra or even its stripes are detected by a system that is sensitive to low-to-moderate spatial frequencies, so named because the distance between changes in illumination spans a relatively wide visual angle. Details, such as the hairs on the zebra, or the letters typed on a page, are handled by systems sensitive to high spatial frequencies, so named because the visual angle between changes in illumination is small. The latter system requires high levels of ambient illumination (daylight) to operate.

To evaluate the function of these systems, contrast sensitivity functions were generated using psychophysical procedures under different levels of illumination (Rice and Gilbert, 1982; Rice and Gilbert, 1990). The subject was shown stimuli comprising dark and light bars. If contrast is high, then the bars appear as black and white and if contrast is low then they appear as shades of gray (or, if using an oscilloscope, shades of green). Wide bars test low spatial frequencies and tap the ability to organize visual stimuli into recognizable shapes. Thin bars, or lines, require high illumination and test the high spatial frequencies that enable one to read. Figure 2 shows contrast sensitivity functions from representative monkeys. The abscissae show the thickness of the vertical bars in cycles/degree. Thick bars are represented on the left, very thin lines on the right. The ordinate is "sensitivity," the inverse of the ratio of illumination coming from the bright bars to the dark spaces between bars. The bottom region of each graph represents sharp contrast: very dark spaces between very bright strips, like a zebra. The top regions represent diffuse illumination with little distinction between bright and dark regions, like gray objects on a foggy day.

Some of the exposed monkeys showed profound deficits, especially in extracting mid- to high frequency information. This represents difficulty seeing, respectively, certain shapes and details. These effects appeared under both high illumination (cone vision) and especially under low illumination (rod vision). This deficit appears to have been irreversible and related to exposure during both pre- and post-natal periods.

Monkeys exposed pre- and postnatally were also examined on a test of temporal visual processing. Here, the temporal frequency at which a flickering sinusoidal grating is perceived as having constant luminance is tested using different frequencies and contrasts in luminance (Rice and Gilbert, 1990). Many exposed animals performed better than controls, especially under low illumination. The authors speculated that the improved performance of animals exposed during brain development to methylmercury could be accounted for by cortical re-organization following selective damage to the small parvocellular neurons that mediate high-resolution

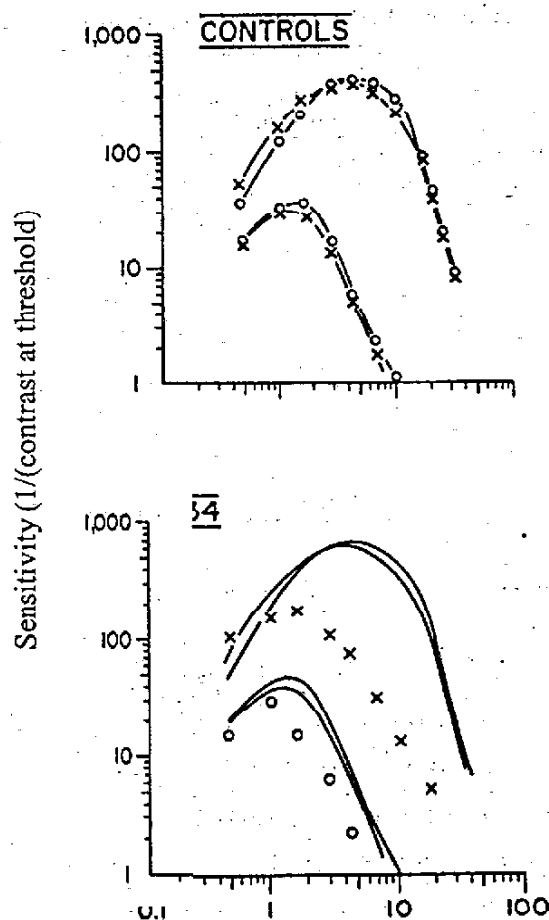


FIG. 2. Contrast sensitivity functions from two unexposed monkeys (top) and from one monkey exposed to methylmercury from birth (bottom). Two sets of curves are shown in each panel; the upper set was taken under high illumination and the lower one under low illumination. The visual stimulus appeared as hazy, vertical bars produced by varying the intensity of illumination sinusoidally. The horizontal axis, spatial frequency, represents the number of vertical bars per degree of visual angle. The left part of the plane represents broad bars, which tap the ability to detect shapes. The right part of the plane represents narrow bars and taps the ability to detect fine detail. Sensitivity is proportional to the inverse of the threshold in contrast between bright and dark regions of the visual field. It is the point at which the monkey cannot distinguish a stimulus that appears to have vertical bars from one that is simply diffuse illumination. (From (Rice and Gilbert, 1982)).

vision. Such re-organization could favor large, magnocellular neurons that mediate low-resolution vision and the visual system's ability to detect flickering stimuli.

In addition to the tests of spatial visual function (contrast sensitivity) and temporal processing of visual information, the monkeys were examined on several other tests of visual function, including peripheral vision, funduscopic examination, and retinoscopy. Apart from myopia in one monkey, retinal examinations and peripheral vision appeared normal. Many monkeys were tested when they were 17 years old or greater, and this is discussed below. This profile sharply distinguishes developmental methylmercury exposure from adult exposure since a hallmark characteristic of adult exposure is a severe narrowing of the visual field (Harada, 1995; Merigan, 1980). Deficits in contrast sensitivity reflect damage higher than the receptor organs and may be related to cortical damage (Greenlee *et al.*, 2000), which would be associated with developmental methylmercury exposure (Rice and Gilbert, 1990).

In a particularly interesting report from the University of Washington, infant monkeys were tested on visual recognition tasks resembling the Fagan task for faces or objects used with children (Gunderson *et al.*, 1986; Gunderson *et al.*, 1988b). For example, in one study (Gunderson *et al.*, 1988b) young monkeys were shown pictures of the faces of other monkeys and the amount of time spent looking at the faces was monitored. This approach exploits the tendency of young monkeys (and children) to spend more time looking at novel faces. It was concluded that the more time the monkey spent looking at a face, the less familiar it appeared.

Methylmercury-exposed monkeys tended to spend more time than controls looking at faces they had seen previously, a result that was interpreted to illustrate a failure of recognition memory or a developmental delay. The studies of Gunderson and colleagues can also be interpreted as revealing a failure in high-order visual processing, an agnosia. This interpretation is consistent with other aspects of methylmercury's neurotoxicity and with the abilities required of the tasks used by Gunderson and colleagues. These tasks appear to involve both subcortical memory systems and cortical information processing (Johnson, 1996). Cortical damage results from chronic, low-level methylmercury exposure during adulthood (Mottet *et al.*, 1987; Vahter *et al.*, 1995) or development (Choi, 1991). With adult exposure at least, damage has been reported in the temporal and occipital lobes, and is especially notable around sulci (Mottet *et al.*, 1987). Facial recognition involves neurons in the superior temporal sulcus (Perrett *et al.*, 1984) and traumatic damage to this area leads to prosopagnosia, an inability to recognize faces (Farah, 1990; Kolb and Wishaw, 1995). Similarly, damage to other regions in the ventral stream, which includes temporal cortex and draws from the same high-spatial-frequency visual functions examined by Rice

and colleagues, impairs the ability to recognize objects visually, even when simpler measures of visual acuity are unaffected (Kolb and Wishaw, 1995). Further support for linking the effects noted by Gunderson and colleagues to visual information processing in the cortex, rather than to recognition memory, derives from the absence of an effect of methylmercury on memory in adult monkeys after developmental methylmercury exposure, (Gilbert *et al.*, 1993a) and the presence of effects on complex spatial vision (Rice and Gilbert, 1982; Rice and Gilbert, 1990).

**Somatosensory Function.** Just as assessing the ability to detect texture in the visual world is an important function of vision, so is detecting texture in the somatosensory world an important component of that system. Rubbing a finger across an object is an effective way of identifying its texture. A coarse object, like stiff corduroy, will deflect the skin by a millimeter or so, and the time between deflections is relatively long (remember, one is moving across a surface, so the distance between corduroy elevations is translated into time between skin deflections). When rubbing a smooth object, like silk or fine sandpaper, the depressions of the skin are smaller and the time between depressions is shorter. This information is translated into the perception of texture and is also an important component of motor control since fine motor acts are modified by sensory feedback.

Somatosensory function was evaluated using vibration sensitivity in old monkeys (15-19 years old), after it was noted that they were clumsy when moving about in their cages and deficient in fine motor control (picking treats out of small holes with their fingers) (Rice, 1996a). To evaluate sensitivity, the hand was secured palm down on a flat surface and a small, vibrating probe was applied to the middle finger. The frequency of vibration (25 to 250 Hz) and its displacement (0.5 to about 20  $\mu\text{m}$ ) were varied systematically. Of five monkeys exposed to methylmercury postnatally to 7 years old, four demonstrated significant impairment of vibration sensitivity and a fifth resembled controls. Of four monkeys exposed *in utero* and postnatally, two showed significant impairment, one modest impairment, and the fourth resembled controls. By impaired, it is meant that the monkey required a greater displacement of the vibrating stimulus before registering a detection. It was argued that the effect was probably related to cortical damage because it was seen over a broad range of vibration frequencies. Damage to the sensory end-organ or peripheral fibers would be expected to affect a narrower range of frequencies.

**Aging.** As the victims of Minamata disease age, it is becoming apparent that their methylmercury exposure is accelerating the aging process in some functional domains (Kinjo *et al.*, 1993). Some monkeys in the Canadian Health Protection cohort were retested on sensory function as they aged. Methylmercury-exposed

monkeys showed enhanced declines in somatosensory (Rice and Gilbert, 1995) and auditory (Rice, 1998a) function. Visual function generally deteriorated when the monkeys were greater than 17 years old, but there was no evidence of an interaction between methylmercury and aging on contrast sensitivity or temporal visual function (detection of flickering stimuli) (Rice and Hayward, 1999a). Some constriction of peripheral vision was noted in the older monkeys, and this constriction had not been detected when they were younger. The constriction of the visual fields was far less severe than has been associated with exposure during adulthood.

### PCBs

Auditory function was tested in adult rats using reflex modification audiometry in rats exposed from gestational day 6 through postnatal day 21 to Aroclor 1254 (0, 1, 4, or 8 mg/kg/day), containing *ortho*- and non-*ortho*-substituted congeners (Goldey *et al.*, 1995). In the adult rats, high-frequency hearing resembled controls but hearing in the low- to mid-range frequencies (in the rat, 1000 Hz) was significantly impaired, opposite to what was reported with methylmercury. Thresholds for the 1000 Hz tone were elevated by 20 to 25 db. This impairment was associated with reductions in circulating thyroid hormone levels, especially thyroxine, that result from developmental exposure to certain PCBs. A profile characterized by selective loss of low auditory frequencies is unusual. A suggestion that this reflected impaired development of the cochlea due to lowered thyroxine levels at a critical stage of development was given credence when thyroxine replacement therapy attenuated the hearing loss (Goldey and Crofton, 1998).

In another study, rats were exposed during gestation to PCB 126, a non-*ortho*-substituted congener (Crofton and Rice, 1999). Like the Aroclor 1254, PCB 126 decreased thyroxine levels (Rice, 1999) and caused a low-frequency hearing loss which, in this study, was extended to a 20 db loss at 0.5 kHz. These studies further suggest that the low-frequency hearing loss is associated with decreases in thyroxine. This does not necessarily imply that low-frequency hearing in humans would also be affected by such compounds. The specific profile of hearing loss would depend upon species differences in the timing of exposure, of thyroxine activity, and of the development of the auditory apparatus. The results do suggest that some hearing loss might occur and that this possibility should be investigated.

### MOTOR EFFECTS

This section will emphasize behavior that reflects motor control, essentially movements that include gait,

posture, or coordinated and guided movements (Brooks, 1986). Locomotor activity, sometimes included in discussions of motor effects, certainly includes such elements as gait but is nonspecific and can be influenced by emotionality, stereotypies, and arousal. As such, it provides an interesting contrast with motor function and is covered briefly at the end of this section.

### Methylmercury

At the high exposure levels seen in Minamata, cerebral palsy, reflective of cortical damage, and cerebellar signs such as nystagmus were noted in a large number of births (Hamada *et al.*, 1993; Harada, 1995). Similar effects have been reported in a monkey exposed *in utero* (Rice, 1983). In Iraq, the most sensitive marker of effect was delayed development of walking (Cox *et al.*, 1989; Cox *et al.*, 1995). The animal literature is consistent with this in that cortical and cerebellar regions in animals exposed developmentally to methylmercury show selective damage, although there are species differences in sensitivity (Choi, 1991; Markowski *et al.*, 1998). Methylmercury exposure in animals has sometimes resulted in abnormal expressions of behavioral development using conventional and advanced tests of motor function, although in some cases the effects may have reflected sensory, and not motor impairment.

Rats exposed to 1.5 or 5 ppm of methylmercury during gestation were trained to press on a small platform with a force that ranged between two predefined limits (Elsner, 1991). The exposed rats showed pronounced impairments on this task. These impairments could reflect either sensory or motor development as the two are sometimes difficult to distinguish. The report also described the appearance of tremor in some of the exposed rats, clearly suggestive of motor involvement, and impairments in swimming ability, also suggestive of a motor deficit. Swimming deficits had previously been observed in animals exposed to 0.5 mg/kg/day during gestation (Elsner *et al.*, 1988b).

Rats exposed to methylmercury on gestational days 6 through 9 were reported to show impaired acquisition of high-rate responding under a differential reinforcement of high-rate (DRH *n:t*) behavior schedule (Bornhausen *et al.*, 1980). Under this arrangement, *n* responses within *t* seconds are required for each reinforcer delivery. Three schedules of food reinforcement, a DRH 2:1 (2 responses within 1 sec), a DRH 4:2, and a DRH 8:4, presented independently and in that order, were used to train these rats to press a lever. Acquisition of high-rate behavior was reported to be impaired in the rats exposed to 10 (and possibly 5)  $\mu\text{g}/\text{kg}/\text{day}$  of methylmercury. Although this result was interpreted to reflect acquisition effects alone, it could also reflect motor dysfunction, given the high-rate behavioral demand of the imposed schedules. The levels

of exposure used in this study were quite low, separating this study from the rest of the literature, as is illustrated below in Figure 6. Unfortunately, the litter did not serve as the statistical unit in this study and an attempt to replicate this effect, using a different implementation of the DRH schedule, was unsuccessful (Rasmussen and Newland, 1999). (Contemporary standards for developmental neurotoxicology studies require that one pup/litter (or one/sex/litter) represent the unit of analysis. That is, 3 rats or 24 rats from three litters would both represent an  $n$  of 3. Littermates can be more similar to one another than they are to unrelated rats so the inclusion of littermates in developmental studies biases the outcome and artificially inflates the degrees of freedom (Buelke-Sam *et al.*, 1985).) Neither brain nor blood mercury levels were reported so direct comparisons using that biomarker of exposure are not possible. Brain mercury levels can be estimated, however, to be well below 1 ppm, a "low" level of exposure (Burbacher *et al.*, 1990a), and probably well below 0.5 ppm, the level reported following an exposure regimen using about 5 times their daily dose (Newland and Reile, 1999a).

As the methylmercury-exposed monkeys in the Canadian Health Protection Branch aged, some appeared clumsy during exercise periods and displayed deficiencies on a test of fine motor control, in which they picked up small treats embedded inside small holes (Rice and Gilbert, 1995). Later tests of somatosensory function, reviewed above, suggested that at least in some of these monkeys the deficits were associated with elevated vibration sensitivity thresholds. Thus, in some monkeys, deficits that appeared to be motor in nature probably resulted from deteriorating proprioception. Other monkeys, also described as impaired in movement, showed no shift in vibration threshold; in these monkeys, at least, motor deficits cannot be ruled out. In aging Minamata patients, declines in certain activities of daily life appeared at an earlier age than in controls from a neighboring village (Kinjo *et al.*, 1993). In a rodent model, high-rate behavior maintained by a DRH schedule of reinforcement declined as animals aged, and for rats exposed during gestation to methylmercury, these declines began at a much younger age than methylmercury-exposed rats (Newland and Rasmussen, 2000).

## PCBs

Exposure to TCDD (0.12  $\mu\text{g}/\text{kg}/\text{day}$  through lactation, after a loading dose of 0.3  $\mu\text{g}/\text{kg}$  2 days before birth) delayed the development of the righting reflex in rats and impaired the ability of mice to remain on a rotating rod (Thiel *et al.*, 1994). Aroclor 1254 also delayed the development of the righting reflex in rats (Overmann *et al.*, 1987), but the non-*ortho*-substituted PCB 126 did not delay this reflex in other rats (Rice, 1999). Fenchlor 42 had

no effect on the developmental landmarks of righting reflex and negative geotaxis in rats (Pantaleoni *et al.*, 1988). Rats exposed to PCB 77 (non-*ortho*-substituted) were given a haloperidol catalepsy test. Acute haloperidol administration can produce a cataleptic posture in which a rat remains fixed in a posture in which it is placed by an experimenter for an extended period of time, a phenomenon usually attributed to the drug's motor effects. Rats exposed to PCB 77 (non-*ortho*-substituted), but not to PCB 47 (*ortho*-substituted), showed prolonged catalepsy, an observation interpreted as being indicative of motor deficits often found with non-*ortho*-substituted PCBs (Hany *et al.*, 1999). Developmental exposure to 32,000  $\mu\text{g}/\text{kg}/\text{day}$  of non-*ortho*-substituted PCB 77 produced a bizarre phenomenon described as "spinning" among some mice in addition to diminished grip strength and ability to traverse a wire rod (Tilson *et al.*, 1979). Taken together, these reports indicate that non-*ortho*-substituted PCBs may impair the maturation or delay the appearance of motor function.

## Locomotor Activity

Most studies of methylmercury exposure in rats have reported little or no effect on locomotor activity (Buelke-Sam *et al.*, 1985; Cuomo *et al.*, 1984; Elsner *et al.*, 1986; Goldey *et al.*, 1994), but some have reported a decrease in locomotor activity (Elsner *et al.*, 1988b) and increases in certain stereotyped behavior patterns (Elsner *et al.*, 1986). TCDD has little or no effect on locomotor activity (Sirikka *et al.*, 1992; Thiel *et al.*, 1994). In one report an increase in locomotor activity was reported in neonates (Eccles and Annau, 1982). As reviewed by Schantz (Schantz, 1999), the non-*ortho*-substituted PCB 77 has increased (Agrawal *et al.*, 1981; Tilson *et al.*, 1979), decreased (Eriksson *et al.*, 1991), or had no effect on (Hany *et al.*, 1999) locomotor activity. PCB 95, a di-*ortho*-substituted PCB, that exhibits some TCDD-like effects, decreased locomotor activity in animals exposed *in utero* and tested as adults (Schantz *et al.*, 1997). Aroclor mixtures often increase locomotor activity, especially in young animals exposed developmentally, but some decreases have been reported later in development (Schantz, 1999).

## LEARNING, DISCRIMINATION, AND MEMORY

The investigation of phenomena that fall into the category of "learning and memory" appear to distinguish effects among methylmercury, *ortho*- and non-*ortho*-substituted PCBs. A functional distinction between the types of behavioral procedures examined under this general heading might help to organize the

literature. Figure 3 illustrates this distinction. As a heuristic, operant behavior (any behavior influenced by its consequences) can be described as a two-term contingency between a response and a consequence (Sidman, 1986). The consequence may increase (or decrease) the likelihood that the response will reoccur. If the probability of response reoccurrence increases, then the consequence is defined as a reinforcer. Negative reinforcers increase the probability of escape and avoidance responses, and punishers decrease the probability of response reoccurrence. This two-term contingency does not actually exist in isolation; instead, it operates within a context to form a three-term contingency (Sidman, 1986). Discrimination describes the extent to which the response occurs only within a specific context. In experimental settings the context is usually a simple stimulus such as light or tone. In studies of remembering, the context is presented and removed before the response is able to occur (McCarthy and Davison, 1991; White and Wixted, 1999). Higher-order, N-term contingencies can describe conditional discriminations in which one three-term contingency is in effect in one setting and a second three-term contingency in another setting, as is exemplified by certain applications of match-to-sample procedures (Carter and Werner, 1978; Newland and Marr, 1985; Sidman, 1986).

#### Reinforcement Contingencies in Operant Behavior.

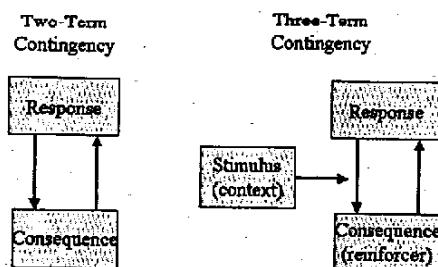


FIG. 3. An illustration of the response-consequence relationship (left) and how a context can modify this (right) as expressed in the three-term contingency of reinforcement. The context can be present as behavior occurs, as in discrimination tasks, or it can be removed before the animal has an opportunity to respond, as in memory tasks.

There is growing recognition in the contemporary behavior analysis literature that response acquisition and contextual control can be viewed as separate, and perhaps independent, processes and that this distinction might have a neural basis (Donahoe *et al.*, 1993; Davison and Nevin, 1999). A functional distinction therefore exists between discrimination, the manifestation of the three-term (or higher-term) contingency, and response acquisition, a manifestation of the two-term contingency. In addition, memory tasks can be viewed as a form of

discrimination task, but with a delay imposed (McCarthy and Davison, 1991; White and Wixted, 1999). Such a distinction may help clarify any differences in developmental neurotoxicity observed with methylmercury and the various PCBs.

## DISCRIMINATION AND MEMORY

### Methylmercury

Methylmercury does not appear to impair discrimination- or memory- related tasks in exposed animals at doses that do not produce generalized impairment. In one report, monkeys were exposed during gestation to 0, 50, 70, or 90  $\mu\text{g}/\text{kg}/\text{day}$  of methylmercury, though the exposure groups were combined for this experiment (Gilbert *et al.*, 1993b). Peak blood mercury levels in the infants ranged from 1 to 2.5 ppm. At 7 to 9 years of age the monkeys were trained under a delayed spatial alternation task using automated training and maintenance procedures, with delays ranging from 0.1 to 15 seconds. Accuracy on this task tended to decline, although only slightly, as the delay interval increased for all animals. However, acquisition was more rapid and the number of errors was generally smaller for the methylmercury-exposed animals than for controls.

This paper (Gilbert *et al.*, 1993b) reports an effect in an unexpected direction and attempts to identify the reasons for it. The ability to monitor many details about behavior is a powerful advantage of the automated procedures used in this study. For example, behavior that spans the entire delay interval, a phenomenon called "rehearsal" and exemplified by the act of repeating a phone number to oneself until it is dialed, often improves accuracy on tests of short-term memory. Monitoring such behavior can identify or rule out some potential reasons for impaired performance. In their implementation of the delayed spatial alternation procedure, the animal was able to continue responding on one of the levers during the delay interval. Although the controls did produce far fewer delay-interval responses, detailed analyses did not indicate that delay-interval responding contributed significantly to the difference between mercury and control animals. A number of animals did not reach the training criterion. For those that did, the delay intervals may have been too short to challenge primate memory. Nevertheless, a similar procedure did detect effects of lead exposure (Rice and Karpinski, 1988) so the procedure should have been sensitive enough to detect impairments had they been present.

Another set of monkeys exposed to 0, 10, 25, or 50  $\mu\text{g}/\text{kg}/\text{day}$  of methylmercury was tested on a nonspatial discrimination-reversal task as juveniles and as adults (Rice, 1992). Once again, no convincing effects of methylmercury were detected in these monkeys,

although they and their cohorts showed effects on sensory function and on fixed-interval schedule performance.

Rodent studies also show a lack of effects on discrimination. In one study (Schreiner *et al.*, 1986) rats were exposed during gestation to methylmercury via maternal drinking water (1.5 and 5 ppm) and tested on a visual discrimination-reversal task at two months of age. While some effects were noted in the high-dose group on the execution of the response, such as latency or likelihood to respond, there were no effects reported on accuracy.

In another study, using a different dosing regimen, rats were exposed to 2 or 6 mg/kg/day on gestation days 6-9 and tested on several discrimination tasks (Buelke-Sam *et al.*, 1985). No effects of methylmercury were reported on olfactory discrimination in pups and only scattered, marginal effects were noted on a discrete trial discrimination task and a discrimination-reversal task, although other developmental endpoints had been affected. Similarly, in a near-replication, rats were exposed to 1, 2, or 4 mg/kg by gavage on gestational day 6 to 15 and although there were effects on reproduction and weight gain, no effects on discrimination were noted in the offspring (Goldey *et al.*, 1994).

## PCBs

Evidence that certain PCB mixtures affect discrimination and remembering in rodents and nonhuman primates has been gathered from a collection of studies using several different procedures. Data from studies using pure congeners, described after the mixtures are presented, support hypotheses about a functional distinction between *ortho*- and *non-ortho*-substituted PCBs.

In a series of studies, female monkeys were exposed to Aroclor mixtures that differed in the number of chlorine atoms (Bowman *et al.*, 1978; Levin *et al.*, 1988; Schantz *et al.*, 1989), studies that are reviewed in (Schantz, 1999; Schantz *et al.*, 1991). For some monkeys, exposure was concurrent with pregnancy and continued through weaning, while for others exposure ended one year or more before conception. The offspring showed deficits on several tasks that involved acquisition or recollection of spatial information. For example, in the delayed spatial alternation procedure (Levin *et al.*, 1988), the experimenter sat behind a shield and presented a monkey with two red blocks. If the monkey picked the left one on the first trial, then a treat was presented and the right block became "correct" on the second trial. The left was correct on the third trial, and so on in an alternating pattern. A delay interval ranging from 5 to 40 seconds separated two consecutive trials. Aroclor 1248-exposed monkeys acquired the conditional discrimination more slowly than controls and accuracy peaked at about 60%, as compared with

75% for controls (Fig 4, top). Performance in control monkeys showed a sharp decline as the delay between trials increased (Figure 4, bottom). No clear delay-related gradient was observed in exposed monkeys; their performance at the shortest delay was also poor. In fact, the distinction between control and PCB-exposed monkeys diminished at the longer delays. The authors concluded that although the procedure involved a delay, the performance deficits were not memory-related, but may have been due to an attentional deficit. If the difference in performance between controls and exposed monkeys had increased with longer delays, then the interpretation would have been that PCB disrupts remembering.

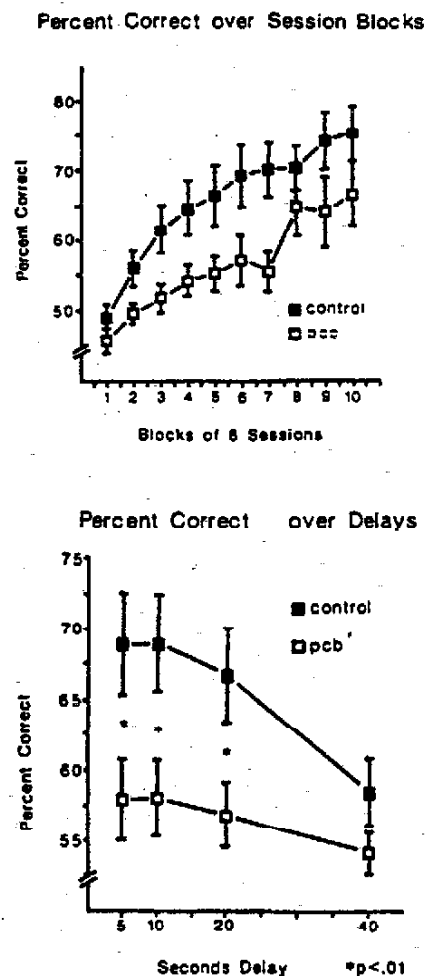


FIG. 4. The top panel shows acquisition of a delayed spatial alternation task in monkeys exposed to Aroclor 1248 (open symbols) and their controls (filled symbols). Note that the acquisition was retarded for the exposed monkeys and terminal performance was lower. The bottom panel shows performance as a function of delay interval between trials. Note that performance between the two exposure groups diminished with increasing delays, suggesting that the effect was not on memory.

The offspring of monkeys exposed before conception to Aroclor 1016 or Aroclor 1248 were trained on discrimination-reversal tasks (Schantz *et al.*, 1991; Schantz *et al.*, 1989). When the discrimination was based on the location of an object, impairment was associated with Aroclor 1248, even though exposure had ended at least one year prior to conception. When discriminations were based on the shape, but not the location of an object, some monkeys showed evidence of improved performance and exposed monkeys required fewer trials to acquire the discrimination. Monkeys born while mothers were exposed to Aroclor 1248 or 1016, *i.e.*, exposure began prior to conception and continued to weaning, also showed impaired spatial reversal learning (Bowman *et al.*, 1978; Schantz *et al.*, 1991; Schantz *et al.*, 1989). The Aroclor 1016-exposed monkeys showed impaired performance only after the stimuli used were the same as had been used on a previous color- and shape-discrimination task (Schantz *et al.*, 1989).

In a study using automated methods of stimulus presentation and behavioral monitoring, a similar pattern of effects was associated with exposure to a mixture of *ortho*-substituted PCBs designed to resemble the constituents found in human breast milk (Rice and Hayward, 1997). Monkeys exposed to the PCB mixture showed consistent impairments on a spatial delayed alternation task. As in the report described above (Levin *et al.*, 1988), the effects were interpreted as a discrimination-learning or performance deficit rather than as a memory deficit because the delay did not amplify the effect of the mixture. When trained on a discrimination task that was based on shape, a non-spatial task, there were no statistically significant group differences, although some treated monkeys had performance deficits well outside the range of controls. In a later report, no effects were discerned on spatial discrimination-reversal tasks in these monkeys (Rice, 1998b).

There is a consistent thread in the results across the studies of PCB mixtures regarding these memory tasks. Impairment associated with PCB exposure is seen on delayed spatial alternation tasks but only occasional effects are found on spatial discrimination-reversal tasks. With non-spatial tasks a range of effects has been noted, including facilitation, absence of effect, and impairment. The Aroclor mixtures used contain *ortho*- and non-*ortho*-substituted PCBs so it is impossible to determine which constituents are responsible for the observed effects and which constituents have no effect. The custom mixture used by Rice and colleagues provide evidence that *ortho*-substituted congeners are toxic components, but pure congeners are required to confirm structure-activity relationships.

In a series of studies aimed at distinguishing the effects of *ortho*- and non-*ortho*-substituted PCBs, Schantz and colleagues exposed rats during development to TCDD or to one of three *ortho*-substituted PCBs (PCB 28, 118, or 153) and examined effects on development, radial

arm maze performance and delayed spatial alternation in a T maze (Schantz *et al.*, 1995; Schantz *et al.*, 1996; Schantz *et al.*, 1997). The mono-*ortho*-substituted PCBs behaved more like the *ortho*, non-dioxin-like congeners. No effects were noted on performance in the radial arm maze, said to be a test of working and reference memory and spatial in nature. Acquisition of performance by females on the delayed spatial alternation T-maze task was impaired in all exposed groups for the three different *ortho*-substituted PCBs.

A fourth *ortho*-substituted congener, PCB 95, described in a separate report (Schantz *et al.*, 1997), produced effects that were more dioxin-like (described below). Rats exposed to PCB 95 showed more rapid acquisition on the radial arm maze task and were indistinguishable from controls in delayed spatial alternation. Thus, three of four *ortho*-substituted PCBs impaired the delayed spatial alternation task but did not affect radial-arm maze performance. The fourth was more "dioxin-like" in its effects even though this congener does not bind to the Ah receptor. It is not clear what the mechanism of this behavioral activity could be, but the different types of effects are reminders that the links between behavior and neural mechanisms of action are not always direct.

In another study, rats were exposed either to TCDD or to non-*ortho*-substituted PCBs (PCB 77 or PCB 126) during development and tested on the same tasks described immediately above (Schantz *et al.*, 1996). Rats exposed to TCDD or to the non-*ortho*-substituted PCBs committed fewer errors on the radial arm maze than controls. No exposure-related differences in the delayed spatial alternation task were found. In another study of exposure to TCDD, the improved accuracy on the radial arm maze was replicated in male rats (Seo *et al.*, 1999). Exposed rats did not differ from controls on a spatially-based discrimination-reversal task, similar to the result seen with the delayed spatial alternation task. However, exposed rats were impaired on the visual (presence versus absence of a low-wattage light) discrimination-reversal task. A similar effect had also been reported with monkeys exposed developmentally to TCDD (Schantz and Bowman, 1989). In that report, spatial discriminations were unaffected, but a nonspatial discrimination task based on the shape of an object was impaired.

In another lab, no convincing effects of PCB 126, a non-*ortho*-substituted congener, on an automated spatial delayed alternation task were reported in rats (Rice, 1999). TCDD had no effect on discrimination learning in rats exposed during development (Thiel *et al.*, 1994) or on passive avoidance (Sirkka *et al.*, 1992). Even in quite complex, higher-order discrimination tasks, aimed at assessing attentional processes more directly, the dioxin-like, non-*ortho*-substituted PCB 126 had no effect on behavior despite effects on other physiological markers (Bushnell and Rice, 1999).

The pattern of effects seen with PCBs and TCDD is complex and difficult to summarize due to the large number of existing congeners, as well as to the differences across studies in dosing regimens, doses tested, developmental stage of exposure, species used, and behavioral procedures employed. Despite these problems, some conclusions can be supported. Developmental exposure to PCB mixtures has pronounced effects on tests of discrimination or remembering. Investigation of specific congeners suggests that the effects are due to *ortho*-substituted congeners in these mixtures and that spatially based discriminations are especially vulnerable, especially when a delay is included. It is not the case, however, that all *ortho*-substituted congeners are behaviorally active in the same way, as discovered with PCB 95 (Schantz *et al.*, 1997). Specific and similar detriments in spatial discrimination, but not in other forms of discrimination, have been observed in monkeys with lesions in the dorsolateral prefrontal cortex, giving rise to a hypothesis that that location is associated with PCB's neurotoxicity (Levin *et al.*, 1992; Schantz, 1999). This hypothesis is very appealing, but until specific information regarding the pattern of damage caused by PCBs becomes available, it must be treated as speculation.

PCBs that are dioxin-like have a different profile of effects. They do not affect spatial discrimination but alter nonspatial discrimination in some procedures. On occasion, effects that are facilitatory have also been reported in several settings. These reports of facilitation should be interpreted with considerable caution. It is possible that performances described as "facilitatory" are not completely understood and could actually be the result of a behavioral deficit. As has been noted by the investigators, certain aberrant or stereotyped behavior patterns could result in "improvements" in accuracy with certain procedures (Schantz, 1999). For example, acquisition of radial arm maze performance could be facilitated by a stereotyped pattern in which the rat simply circles through the maze selecting the adjacent arm, a pattern not seen in control rats. The important points here are that these compounds have behavioral effects that are distinguishable from controls, even if they appear to be facilitatory, that there is no known medical benefit to them, and that there is toxicity associated with brain as well as other organ systems (Safe, 1990; Van den Berg *et al.*, 1998).

## LEARNING

All learning tasks require some form of discrimination and remembering, at least in the sense that behavior on one day reflects acquisition of a response-consequence relationship that occurred on a previous day of training. Moreover, memory tasks such as those described above

require the acquisition of a novel response-consequence relationship. Tasks differ, however, in the degree to which they emphasize the different behavioral processes. There is some evidence that methylmercury exposure can alter the acquisition of a response-consequence relationship in procedures that minimize the role of short-term remembering, but further work is needed.

## Methylmercury

In one experiment, three squirrel monkeys were exposed to methylmercury during gestation such that maternal blood levels were 0.8 - 0.9 ppm. As juveniles, the offspring were trained to lever-press under a procedure called a *concurrent random-interval random-interval* schedule of reinforcement (Newland *et al.*, 1994). In brief, a monkey sat in a chair facing two response levers and a reinforcer pellet dispenser. Pressing the left lever produced a food pellet after a random interval, averaging one food pellet every 30 seconds, expired. Pressing the right lever produced food pellets at the same rate. So the overall reinforcer rate associated with each lever was 2/min. This is called a Concurrent Random Interval 30" Random Interval 30" (*conc RI 30" RI 30"*) schedule of food reinforcement. After behavior stabilized under this arrangement, the *conc RI 30" RI 30"* was replaced by a *conc RI 60" RI 15"* schedule, thus changing the individual reinforcement rates such that the right side produced food four times more often than the left side.

Figure 5 shows what happened under this arrangement. After the schedule changed such that most reinforcers followed responses on the right lever, the responding of control monkeys gradually shifted so that eventually most behavior occurred on the richer, right lever. This finding is consistent with that of *steady-state* performance documented in a large literature describing the behavior of human and nonhuman subjects under concurrent schedules of reinforcement (Davison and McCarthy, 1988; de Villiers, 1977; Kollins *et al.*, 1997). With concurrent random-interval random-interval (or variable-interval variable-interval) arrangements, the percent of the total number of responses (relative responses) made on a lever approximately matches the percent of the total number of reinforcers (relative reinforcers) derived from that lever. This phenomenon has been observed across a wide range of settings, species, and experimental parameters. Less is known about concurrent schedule behavior during transition states.

Initial training of the exposed and control monkeys on the concurrent schedule had not revealed any exposure-related differences in behavior. However, exposure-related differences were observed after behavior stabilized and the reinforcement contingencies changed from a stable baseline. As seen in the figure (bottom panel), relative responses did not track relative

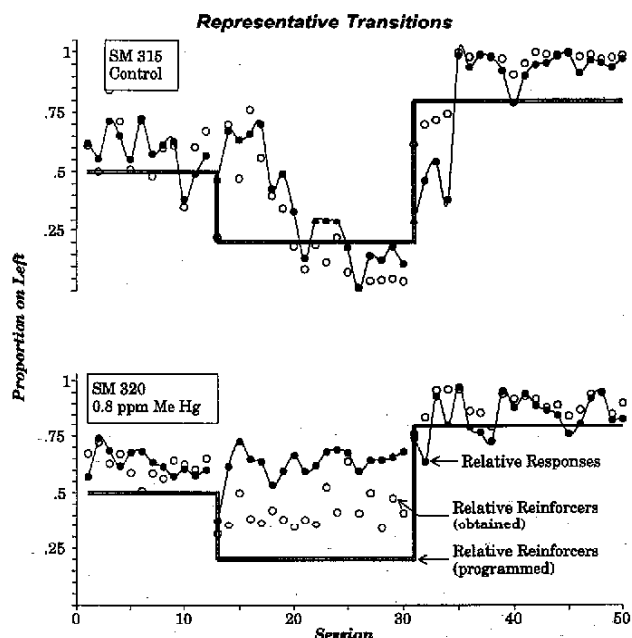


FIG. 5. Representative transitions for a control monkey and one exposed to methylmercury during gestation (adapted from (Newland and Reile, 1999b; Newland *et al.*, 1994)). Symbols represent summaries of performance for individual 30 minute sessions. Relative response rate and obtained and programmed reinforcement rates are shown for two transitions. In the top panel the change in behavior and obtained reinforcement rates lagged programmed rates by 4, 30-min sessions. For the exposed monkey, behavior showed little sensitivity to programmed or obtained reinforcement rates during the first transition.

reinforcers following the change from *conc RI 30" RI 30"* to *conc RI 60" RI 15"* for a methylmercury-exposed monkey. Even though reinforcers came from the right lever, most responses occurred on the left one. Analyses of this performance suggested that the behavior of the methylmercury monkeys was relatively insensitive to its consequences and heavily biased. This effect was detectable in part because of the sensitivity afforded by experimental arrangements that permit the quantification of choice under a concurrent schedule (Newland and Reile, 1999b).

Others have reported disruptions in learning of response-consequence relationships in rodent species, although the doses evaluated were much higher in those studies and the exposure conditions were different. In one study (Hughes and Annau, 1976) mice were exposed to 0, 1, 2, 3, 5, or 10 mg/kg of methylmercury on day 8 of gestation and underwent behavioral testing as adults. Their mothers (the F0 generation) and their offspring (the F2 generation) were also evaluated on some behavioral tests. One such test investigated two-way shuttlebox avoidance. The general shuttlebox procedure involves placing the mouse in a small chamber with an electrifiable floor. At first, half of the floor becomes electrified

and the mouse can escape the shock by moving to the other half, an escape response. After a period of time, that other half becomes electrified instead, and the mouse can escape by moving again. This alternation continues through the experiment. Offspring exposed to 3 or 5 mg/kg of methylmercury required twice as many trials to acquire this task as controls. Higher doses had reproductive effects and were not evaluated on this task. Exposed mothers were indistinguishable from controls, and the F2 generation also appeared unaffected. A similar result was reported using rats in another experiment (Eccles and Annau, 1982). There did not appear to be sensory effects that diminished shock sensitivity *per se*, as the exposed and control mice performed the same on a conditioned suppression procedure, which involves the presentation of response-independent shock.

## PCBs

Greater avoidance, perhaps facilitated avoidance, was observed in rats exposed to a PCB mixture (Lilienthal *et al.*, 1990), an effect that seemed related to prenatal, but not postnatal, exposure (Lilienthal and Winneke, 1991). With a different task, step down passive avoidance performance was impaired in rats exposed to PCB 77, a non-*ortho*-substituted PCB, but not in rats exposed to the di-*ortho*-substituted PCB 47 (Hany *et al.*, 1999).

A pair of studies examined the effects of a PCB mixture containing many *ortho*-substituted congeners, using monkeys, and a non-*ortho*-substituted congener (PCB 126), using rats, on *conc RI RI* responding (Rice and Hayward, 1999b; Rice and Hayward, 1999c). As previously noted, this concurrent schedule procedure permits an assessment of the acquisition of a response-consequence relationship with relatively little contribution from discrimination processes. In these studies, initial acquisition of concurrent schedule performance (after lever-press training) was examined. Later, transitions from the initial concurrent schedule to a second and then to a third schedule were examined. The behavior of monkeys exposed to the mixture of *ortho*-substituted congeners was indistinguishable from controls (Rice and Hayward, 1999c). The non-*ortho*-substituted PCB 126, however, affected the acquisition and the rate of a transition from one concurrent schedule to the next in rats. The latter effect was reflected in the slope of the function describing the behavioral change from one schedule to another. For females, this slope was close to zero, indicating no behavior change, and for males, a negative slope indicated that behavior changed in the *opposite* direction from the source of reinforcement. These and other measures, like bias and, to a lesser degree, final performance before the next transition, suggested that behavior was relatively insensitive to the source of reinforcement. Steady-state performance was

also affected by the highest dose of PCB 126 (1  $\mu\text{g}/\text{kg}/\text{day}$ ) but not by the lowest dose (0.25  $\mu\text{g}/\text{kg}/\text{day}$ ). Thus, acquisition and behavior-change were affected, but effects on steady-state performance were minimal.

Subsequently these rats were tested on a progressive-ratio schedule in which the number of responses required for a reinforcer is small, initially, but increases incrementally until the animal stops responding. Progressive ratios are frequently used to assess the efficacy of reinforcers, such as abused drugs (Griffiths *et al.*, 1977). Exposure to PCB 126 did not diminish response rate, change the pattern of interresponse times, or alter the number of responses produced, indicating that food was an effective reinforcer for all rats and that the PCB did not induce motor disruptions impairing the rats' abilities to respond. Some effects were noted on post-reinforcer pausing and the pattern of acquisition but, as the authors pointed out, the relevance of these effects was not clear. The rats in this study had been examined on several other tasks, including delayed spatial alternation and multiple fixed-ratio fixed-interval schedules of reinforcement. Despite evidence of retarded weight gain and altered thyroxine levels, there were no convincing effects noted on those behavioral tests.

When monkeys were tested with the PCB mixture of *ortho*-substituted congeners, no effect on progressive-ratio performance was observed. Exposed monkeys were indistinguishable from controls in this procedure, although they were impaired on a delayed spatial alternation task and differed from controls in fixed-interval schedule performance.

Results from the experiments discussed in this section and in the previous section on methylmercury show that developmental exposure to non-*ortho*-substituted PCBs or to methylmercury can retard the acquisition of a response-consequence relationship, even if it has minimal effects on discrimination learning or remembering. This general finding suggests that there was reduced sensitivity of behavior to its consequences in the exposed subjects.

## SCHEDULE-CONTROLLED BEHAVIOR

Most of the behavior described in this review is operant behavior under the control of a schedule of reinforcement. Operant behavior is any behavior, lever pressing or even very complex response units, whose appearance is influenced by previous consequences (Marr, 1979; Newland, 1995; Newland and Marr, 1985). A schedule of reinforcement is simply a way to describe the contextual and response contingencies under which consequences are delivered (Zeiler, 1977; Zeiler, 1984). Thus, operant behavior under some schedule of reinforcement is studied using most of the procedures reviewed in this

paper, including spatial alternation, match-to-sample, concurrent schedules, and discrimination reversal to name just a few. Reflexive behavior, such as the pre-pulse inhibition is clearly not operant behavior. Locomotor activity could be, but usually consequences are not controlled in those procedures.

As used in neurobehavioral toxicology, "schedule-controlled operant behavior" typically refers to behavior under one of a limited subset of technically defined reinforcement schedules, usually fixed-interval (FI), fixed-ratio (FR), and differential-reinforcement-of low-rate (DRL) schedules. Because of the role that these specific reinforcement schedules play in neurotoxicology, these are the ones that will be included in this section.

The reinforcement schedules studied have several features that make them well-suited for characterizing neurotoxicants. First, they show great species generality (Morse and Kelleher, 1977; Weiss and Cory-Slechta, 1994). DRL, FI, and FR schedules produce patterns that differ greatly among themselves. Schedule-typical patterns are seen in rodents, birds, monkeys, apes, and (when the role of language is minimized) humans. Thus, we know what to expect of behavior under control conditions, and therefore have an initial check on the competence of the implementation of a schedule before exposure conditions are analyzed. Second, behavior under these technical schedules has been well characterized. An extensive literature has identified important determinants of behavior under these schedules (Zeiler, 1977; Zeiler, 1984). Third, these schedules have been used to identify drug effects, so the effects of drugs from a range of pharmacological classes have already been characterized (Seiden and Dykstra, 1977). This contributes to an understanding of sensitivity as well as characterization of neurotoxicant effects insofar as the profile of effects produced by a toxic chemical resembles that produced by a drug whose neural actions are known. Fourth, they *approach* being an apical test. It is true that, in a sense, all behavior is apical in that it represents the integrated activity of nervous system function. Nevertheless, many behavioral procedures, such as those described in other sections, are designed to isolate specific sensory, motor, memory, or other behavioral functions and can be quite specific. While the production of schedule-typical patterns of responding can be specific to the reinforcement contingencies, they cannot be traced to specific nervous system activities as can, say, contrast sensitivity function. This having been noted, differential effects can be quite instructive. Deficits in FR responding may reflect motivational or motor capabilities (Newland, 1997; Paule, 1990) while alterations in DRL responding reflect impaired temporal control over behavior, sometimes interpreted as attentional deficits. The FI schedule taps a range of functions including inhibitory processes, maximal response rates, and the temporal organization of behavior (Rice, 1988; Weiss and Cory-Slechta, 1994).

## Methylmercury

Under a fixed-interval  $t$  seconds (FI  $t$ ) schedule of reinforcement, a reinforcer is delivered for the first response that occurs after a period of time,  $t$ , has elapsed. A response is required, but only one. This simple-to-describe schedule gives rise to a remarkably complex pattern of behavior that is extremely replicable even if not yet completely understood (Zeiler, 1984). FI schedules typically produce a large number of responses, although the exact number can vary widely on an interval-by-interval basis. Responding is relatively unlikely to occur early in an interval (e.g., the first 100 seconds of an FI 300" schedule). If responding does occur during the early period of an interval, then response rates tend to be low and individual responses are separated by long interresponse times. Late in the interval (e.g., the last 30 sec of an FI 300" schedule) response probabilities are high; response rates tend to be much higher and, correspondingly, interresponse times are short. This temporal pattern can be described by several measures, including the relatively simple measure, the quarter-life—the point in the interval at which one fourth of the responses have occurred. A quarter life greater than 25% of the interval's duration indicates that responding tends to occur late in the interval. A short quarter life can be produced by impaired temporal control over behavior, in which responding tends to occur at a steady rate through the interval, or by the appearance of response bursts throughout the interval.

Methylmercury disrupts the temporal pattern seen in FI-maintained behavior in a specific way that distinguishes it from lead and many behaviorally active drugs (Gilbert *et al.*, 1996; Rice, 1992). In one report, juvenile monkeys were exposed developmentally to methylmercury (10 to 50  $\mu\text{g}/\text{kg}/\text{day}$  pre- and postnatally) and then trained to respond under a fixed-interval schedule. The exposed monkeys tended to respond earlier in the interval, but their local response rates through the interval were indistinguishable from those of controls. In contrast, lead's effect on FI performance resembles that of psychomotor stimulants: responding early in the interval is characterized by high-rate response bursts. Disruptions in temporal patterns of behavior, with methylmercury, have also been reported in adult monkeys (Gilbert *et al.*, 1996), but in that study quarter life was actually larger in exposed males, indicating sharper temporal patterning. Quarter-lives were shorter than controls in the exposed females.

The DRL- $t$  schedule specifically targets temporal control over responding. Under this schedule, a reinforcer is delivered when two responses are separated by  $t$  seconds. Thus, under a DRL 10" schedule the animal must press a lever, do something else for 10 seconds, and then press the lever again. Methylmercury-exposed rats are indistinguishable from controls in this arrangement (Eccles and Annau, 1982). Thus, methylmercury does not

seem to affect behavior under a DRL schedule. By contrast, animals exposed to lead or to psychomotor stimulants respond similarly under DRL and FI schedules. Lead, like stimulants, disrupts temporal control and produces high-rate bursts under the DRL and FI schedules in rodents and monkeys (Cory-Slechta, 1990; Cory-Slechta *et al.*, 1996; Mele *et al.*, 1984; Michaelis *et al.*, 1987; Rice and Gilbert, 1985; Sanger *et al.*, 1974). Methylmercury did neither of these things. Thus, methylmercury can be distinguished firmly from lead in its effects on behavior under these two schedule arrangements, and it can also be distinguished from certain PCBs as well, as seen immediately below.

## PCBs

Monkeys exposed developmentally to a PCB mixture containing *ortho*-substituted congeners found in human breast milk differed from controls in their performance on an FI 6 minute schedule of juice reinforcement (Rice, 1997). The effect was associated with the occurrence of more interresponse times less than 5", which affected measures of temporal control produced by the FI schedule. As noted above, methylmercury disrupted measures of temporal control in different ways.

In another study, the behavior of monkeys exposed developmentally to the Aroclor 1248 mixture was maintained under a range of FI schedules (FI 30", FI 60", FI 300", FI 600") in order to evaluate parametrically the role of FI schedule length in isolating effects of PCB exposure (Mele *et al.*, 1986). At the longer FI values, monkeys exposed to the PCB mixture showed disruptions in the temporal pattern of responding and also showed some elevations in response rate. The former effect was statistically significant. The latter effect was apparent in the graphical display but was not statistically significant, probably because of the presence of a single outlier in the control group. It was unclear whether this effect resulted from a disruption in temporal control over behavior or from an elevation in the number of short interresponse times, as reported in the study described above (Rice, 1997). These exposed monkeys continued to respond at a very high rate in "reinforcement omission" testing, a procedure designed specifically to evaluate temporal control. Also, these monkeys did not differ from controls in open field activity. In contrast, lead and psychomotor stimulants show elevated activity on such tests.

In yet another study, rats exposed to a different PCB mixture (Clophen A-30) also showed disrupted performance under an FI 30" schedule of reinforcement. Specifically, a higher number of responses occurred early in the FI intervals (Lilienthal *et al.*, 1990).

Monkeys exposed to a mixture containing *ortho*-substituted PCBs showed pronounced effects on

performance with a DRL-30" schedule of reinforcement (Rice, 1998b). Recall that the DRL schedule requires an animal to respond, do something else (for 30 seconds in this case), and then respond again. Here, the exposed monkeys showed interresponse times of 10 sec or less more often than they showed interresponse times that were 30 sec or greater, and there was little indication of improvement over the course of the experiment.

An attempt to isolate the contributions of different congeners to the FI pattern was undertaken by exposing rats to PCB 153 (*ortho*-substituted) or PCB 126 (*non-ortho*-substituted) (Holene *et al.*, 1998). Using an FI 2 min schedule, they examined the temporal pattern of behavior, as well as interresponse times during different portions of the interval. Both congeners elevated overall rates of responding. Only the *ortho*-substituted congener, PCB 153, did so by shortening interresponse times, *i.e.*, by producing short interresponse-times early in the FI. The *non-ortho*-substituted isomer, PCB 126, did so without producing these short interresponse times as did methylmercury. Unfortunately, it was not made clear that the litter was used as the statistical unit. In another study, rats were

exposed to PCB 126 and no effects were noted on behavior under either a fixed-interval or a DRL schedule of reinforcement (Rice and Hayward, 1998). Another *ortho*-substituted isomer, PCB 118, and the *non-ortho*-substituted PCB 126 isomer both altered performance on a random-ratio schedule of reinforcement (Holene *et al.*, 1995), but it appears that the litter did not serve as the statistical unit in this experiment either. Progressive ratio performance, described above, was not affected by exposure to PCB 126 (Rice and Hayward, 1999b).

## SUMMARY OF EFFECTS

Table 1 summarizes the effects described above. While it may be possible to identify profiles of effects, the summary must be viewed as tentative because of the incompleteness of the data base.

Developmental exposure to methylmercury affects higher-order sensory function. The effect is detectable with procedures that tap the organization of complex stimulus information. This is noted in visual, sensory

TABLE 1. Comparison of Methylmercury's and PCBs' Effects as Seen in Animal Studies.

General System	Specific function	MeHg	PCBs		
			<i>Non-ortho</i>	<i>Ortho</i>	Mixture
Sensory	Audition (operant testing)	↓↓			
	Somatosensory	↓			
	Visual Contrast Sensitivity	↓↓			
	Visual temporal processing	↑			
	Retinal function	0			
	Audition (reflex modification)	0	↓↓		↓↓
Motor		↓↓	↓↓	0	
Discrimination and memory	Delayed Spatial Alternation	0↑	00	↓↓	↓↓
	Spatial discrimination reversal		0	↓↓	↑↓
	Nonspatial discrimination reversal	00	↓		↓↓
	Radial Arm Maze		↑↑	00	
Learning	Concurrent Schedule/transition	↓	↓		0
	Escape/avoidance	↓	↓		↓
SCOB	Temporal pattern under FI	↓↓	↓	↓	↓
	Short IRTs under FI	0	0	↓↓	↓↓
	DRL	0		0	↓
	FR/progressive ratio		0		
	Random ratio		↓	↓	

- ↓ indicates a deficit.  
 ↓↓ indicates a deficit that was replicated.  
 0 indicates that the function was tested more than once, and unaffected; 00 indicates replication.  
 ↑ indicates facilitation or improvement.  
 ↑↑ indicates facilitation that was replicated.  
 ↓ 0, ↑↓ indicate inconsistencies across studies.  
 An empty cell indicates that the function has not been examined.

and, in older monkeys, somatosensory systems. The nature of the effect is consistent with a cortical locus of damage. Procedures that evaluate the function of the cochlea or auditory nerve did not detect a methylmercury effect.

Unfortunately, higher order sensory endpoints have not been tested with PCBs, but it is evident that exposure to certain PCBs alters hearing, probably by acting on peripheral components of hearing. The mechanism may be through disruption of the development of the sensory organ due to disrupted thyroxine levels during development. *Ortho*-substituted PCBs have not been examined with this procedure as of this writing.

Methylmercury appears to have motor effects in rodent studies at high doses. It also appears that the non-*ortho*-substituted PCBs have motor effects and that the *ortho*-substituted ones do not, but the full picture from the PCBs is not clear yet. Interestingly, this conclusion contrasts with the effects of the different toxicants on locomotor activity. As reviewed by Schantz, studies of mixtures often, but not always, report increases in locomotor activity. Non-*ortho*-substituted PCBs have little effect or decrease locomotor activity. Methylmercury has little effect or decreases locomotor activity.

*Ortho*-substituted PCBs disrupt memory and certain discriminations in rodents, especially when the discriminations are spatially based. Methylmercury and non-*ortho*-substituted PCBs do not have this effect. Acquisition of response-consequence relationships, involving either positive reinforcement or avoidance, is affected by methylmercury and non-*ortho*-substituted PCBs.

Methylmercury and non-*ortho*-substituted PCBs disrupt the temporal pattern of fixed-interval schedule performance, but without a substantially elevated number of short interresponse times. *Ortho*-substituted PCBs do appear to produce these short interresponse times.

## DOSE-EFFECT RELATIONSHIPS

### Methylmercury

Figure 6 shows Lowest Observed Effect Levels (LOELs) from selected reports of developmental neurotoxicity of methylmercury, including studies reviewed above. The vertical axis shows the endpoints listed and the horizontal axis shows the lowest dose at which an endpoint was affected in a particular study. Open symbols come from studies using rats and filled symbols from those using nonhuman primates. With a single exception, the Bornhausen study, primates were more sensitive than rodents by about one order of magnitude. Overall, sensory/motor effects appeared the most sensitive, and in neither species were discrimination processes impaired.

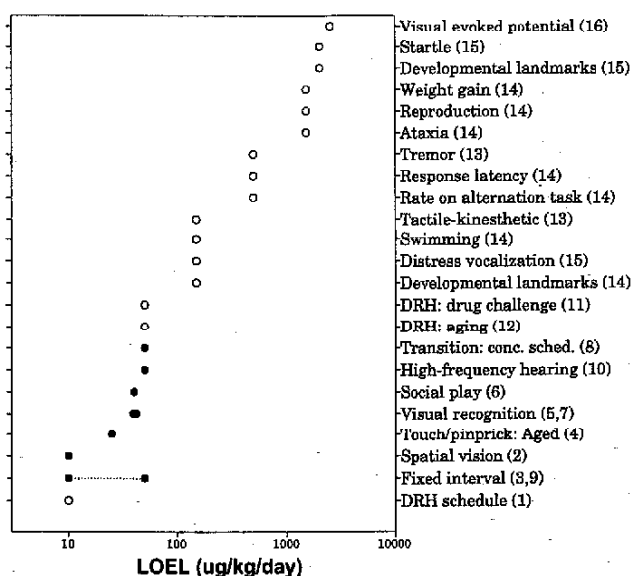


FIG. 6. The dose at which selective effects of developmental exposure to methylmercury appeared in primates (filled circles) and rats (open circles). Dose is expressed as  $\mu\text{g}/\text{kg}/\text{day}$  as estimated or as reported in the study. "Effect" is the lowest dose at which that effect appeared. The studies represented are: 1 (Bornhausen *et al.*, 1980); 2 (Rice and Gilbert, 1982); 3 (Rice, 1992); 4 (Rice and Gilbert, 1995); 5 (Gunderson *et al.*, 1988b); 6 (Burbacher *et al.*, 1990b); 7 (Gunderson *et al.*, 1986); 8 (Newland *et al.*, 1994); 9 (Gilbert *et al.*, 1996); 10 (Rice and Gilbert, 1992); 11 (Rasmussen and Newland, in press); 12 (Newland and Rasmussen, 2000); 13 (Elsner, 1991); 14 (Elsner *et al.*, 1986); 15 (Elsner *et al.*, 1990); 16 (Zenick, 1974)

The greater sensitivity of nonhuman primates can be accounted for by binding characteristics of methylmercury in blood and the nature of the behavioral procedures used. Methylmercury binds avidly to sulfur in red blood cells, and the ratio of red blood cells to plasma in rats is about 145:1, as compared with 17:1 to 25:1 for different primate species (Magos, 1987). After several acute administrations of methylmercury to a pregnant female, the brain:blood ratio in offspring is about 1:14 in rats (Magos, 1987). If exposure begins long before mating and continues, via drinking water, through gestation, this ratio drops to about 1:7 (Newland and Reile, 1999a). By comparison, this ratio ranges from about 2:1 to 4:1 in nonhuman primates (Magos, 1987). In either species this ratio increases over time after exposure ends, reflecting accumulation of inorganic mercury that cannot readily leave the brain (Aschner and Aschner, 1990; Vahter *et al.*, 1994; Vahter *et al.*, 1995).

Many of the studies using rodents, especially the earlier studies, used high doses administered for a few days during gestation and used only screening tests. Some of the more recent studies (Elsner *et al.*, 1988a; Elsner *et al.*, 1988b; Newland and Rasmussen, 2000; Newland and Reile, 1999a; Rasmussen and Newland, in press) used chronic exposure, beginning before mating, in addition to advanced, focused behavioral procedures and discovered more subtle effects at lower exposure levels.

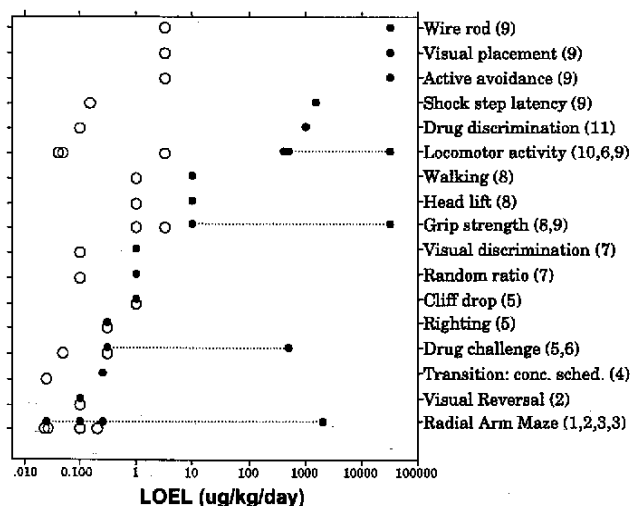


FIG. 7. The dose at which selective effects of TCDD or non-*ortho*-substituted PCBs affect behavior. The filled circles show doses as actually administered. Open circles show conversion to Toxic Equivalencies using factors described in (Van den Berg *et al.*, 1998). The studies represented are: 1 (Schantz *et al.*, 1995); 2 (Seo *et al.*, 1999); 3 (Schantz *et al.*, 1996); 4 (Rice and Hayward, 1999b); 5 (Thiel *et al.*, 1994); 6 (Hany *et al.*, 1999); 7 (Holene *et al.*, 1995); 8 (Bernhoft *et al.*, 1994); 9 (Tilson *et al.*, 1979); 10 (Eriksson *et al.*, 1991); 11 (Lilienthal *et al.*, 1997)

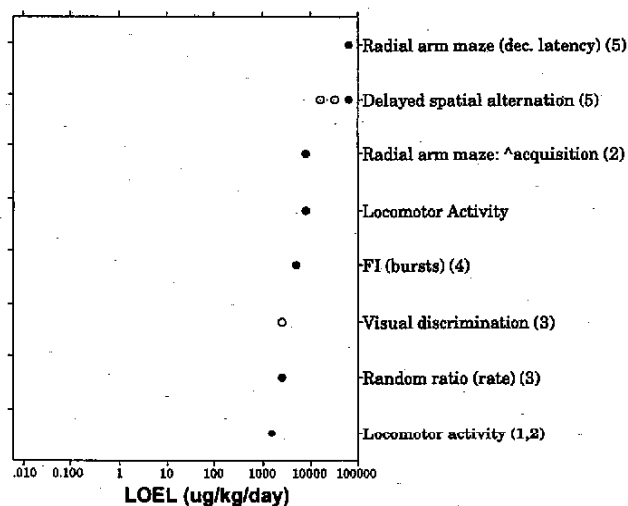


FIG. 8. The dose at which selective effects of *ortho*-substituted PCBs affect behavior. Scale as in Figure 7. 1 (Hany *et al.*, 1999); 2 (Holene *et al.*, 1995); 3 (Holene *et al.*, 1998); 4 (Wong *et al.*, 1997); 5 (Schantz *et al.*, 1995). Open symbols represent use of mono-*ortho*-substituted PCBs

## PCBs

The large number of PCB mixtures and congeners can make it difficult to arrive at a meaningful dose-effect relationship for these compounds, a necessary step in arriving at reference doses. An earlier attempt arrived at estimates that varied by as much as four orders of magnitude depending on the methods used (Tilson *et al.*,

1990). The development of Toxic Equivalency Factors (TEFs) and a better understanding of the separate effects of the coplanar, non-*ortho*- and the non-coplanar, *ortho*-substituted PCBs reduces this range of variability.

Figure 7 shows an attempt to describe a dose-effect relationship across studies for non-*ortho*-substituted PCBs, structured as in Figure 6 for methylmercury. Only rodent species have been used in investigations of specific congeners. Dose is expressed as  $\mu\text{g}/\text{kg}/\text{day}$  as best as it could be estimated from individual reports. Only studies that included gestational exposure are included. The filled symbols represent dose of the particular congener tested. The range of effects spans six orders of magnitude when expressed this way. The open symbols show the same effects expressed as Toxic Equivalencies, doses arrived at after using Toxic Equivalency Factors recently published for mammals (Van den Berg *et al.*, 1998), which represents some changes in TEFs as compared with an earlier list (Safe, 1990). Three non-*ortho*-substituted compounds are represented in the figure with Toxic Equivalency Factors as follows: 1.0 for TCDD, 0.1 for PCB 126, and 0.0001 for PCB 77. This conversion narrows the range of variability to only three orders of magnitude for most endpoints. This reduction of the range is consistent with hypotheses that the behavioral effects of these compounds are related to their structural resemblance to the planar TCDD molecule.

Figure 8 shows an attempt to describe a dose-effect relationship with *ortho*-substituted PCBs similar to the previous two figures. No Toxic Equivalency Factor has been applied in this figure. With these compounds, even though four congeners are represented (PCB 47, PCB 95, PCB 118, PCB 153) the range of doses is relatively narrow. The ambiguity in classifying the mono-*ortho*-substituted PCBs is illustrated in the actions of PCB 118. PCB 118 impaired visual discrimination and responding under a ratio schedule of reinforcement (Holene *et al.*, 1995), effects resembling the non-*ortho*-substituted PCBs. However, it and another mono-*ortho*-substituted PCB (PCB 28) affected delayed alternation in a T-maze and thus were *ortho*-like.

Note that the scaling of the horizontal axis is the same as in Figure 7. The effective doses are far to the right of the dose-effect curve determined for non-*ortho*-substituted compounds, at least when expressed after applying a TCDD Toxic Equivalency Factor. Two of the PCBs represented in Figure 8 are mono-*ortho*-substituted and their planarity lies between that of the non-*ortho*-substituted and di-*ortho*-substituted PCBs. They are poor at occupying the Ah receptor and have effects at exposure levels similar to those seen with the *ortho*-substituted PCBs. These two notwithstanding, the distinction between the two classes of PCBs, the non-*ortho*-substituted/dioxin-like/coplanar and the *ortho*-substituted/non-coplanar PCBs, seems to be qualitative rather than merely dose-related, because the profiles of effects in Table 1 differ so sharply.

It might be argued that regulating PCBs according to their dioxin-like Toxic Equivalency Factors will confer protection because of the high levels of exposure required for the *ortho*-substituted effects to appear. Figures 7 and 8 do not disconfirm this claim, at least with respect to specific congeners, but such an approach may not completely account for the "weathering" of mixtures as they wend their way through ecosystems (Giesy and Kannan, 1998). The distribution of congeners in a technical mixture, such as Aroclor 1254, differs from the distribution of congeners seen in various tissues because of differences in absorption and retention associated with chlorine content. Lightly chlorinated congeners are more readily absorbed but are less likely to be retained (Giesy and Kannan, 1998). This process can cause biomagnification of heavily chlorinated compounds by as much as two orders of magnitude, an effect seen with both *ortho*- and non-*ortho*-substituted congeners (Giesy and Kannan, 1998). Insofar as the heavily-chlorinated congeners tend to have some *ortho*-substitution, the magnification may favor the *ortho*-substituted congeners by one or two orders of magnitude (Giesy and Kannan, 1998). It is noteworthy that the PCB mixture studied by Rice and chosen to match PCBs found in human breast milk was made up of *ortho*-substituted PCBs. The dose-effect curves suggest that protection would still be afforded by considering only non-*ortho*-substituted PCBs, but the margin of safety narrows.

### SUMMARY

This review focused on the effects of developmental exposure to methylmercury or various PCB mixtures or congeners as expressed in adult animals. Some important classes of effects have been omitted in the direct comparisons, in part to preserve focus and in part because comparisons between methylmercury and PCBs were not available. For example, in an interesting series of studies out of the University of Washington, monkeys have shown deficits or developmental delays on several tests of social and cognitive development associated with methylmercury exposure (Burbacher *et al.*, 1990a; Burbacher *et al.*, 1990b; Gunderson *et al.*, 1988a; Gunderson *et al.*, 1988b), but no comparable examination of PCB-related effects could be located.

The hypothesis that the planarity of the PCB congener is related to its neurotoxicity is an appealing one that serves to organize the neurotoxicity of this complex array of congeners. It also serves to link neural effects of the PCBs with the effects seen in specific functional domains. It is clear that developmental exposure to PCBs and TCDD have effects that are manifested as behavior changes, and it is evident that the profile of effects is influenced by the structure of the molecule, *i.e.* whether it can assume a coplanar shape. The presence of behavioral effects does not

necessarily indicate that the effects are directly neural. Hypotheses about TCDD-like effects on thyroid hormones and the Ah receptor imply that endocrinologically based effects, perhaps related to endocrine effects on neural development, can be manifested in the adult behavior of developmentally exposed animals. *Ortho*-substituted PCBs seem to have some task-specific effects, especially on spatially-based discriminations, that distinguish them from the TCDD-like compounds. These profiles support hypotheses that the structural distinction between non-*ortho*-substituted and *ortho*-substituted congeners is an important one, but this distinction does not account for all PCB effects. Despite its appeal, it would be remarkable, given the complexity of the nervous system and of the various PCB congeners, if the hypothesis about the role of PCB structure held under all conditions. Some evidence reported above suggests that there may be exceptions, at least with PCB 95 (Schantz *et al.*, 1997).

Mechanistic hypotheses regarding the distinction between non-*ortho*-substituted and *ortho*-substituted PCBs could also imply an interaction between PCB and methylmercury exposure. A central feature of the hypothesis that *ortho*- and non-*ortho*-substitution have different profiles of toxicity is that developmental exposure to *ortho*-substituted PCBs disrupts the functioning of dopamine (and possibly other monoamine) systems, perhaps by altering calcium homeostasis (Seegal, 1995; Seegal *et al.*, 1991) or the ryanodine receptor in such a way that it enhances calcium homeostasis in presynaptic neurons (Schantz *et al.*, 1997; Wong *et al.*, 1997). Methylmercury can increase dopamine concentrations *in vitro*, perhaps due to enhanced release (Kalisch and Racz, 1996; Winneke, 1992) or retarded clearance from the synapse (Chakrabarti *et al.*, 1998; O'Kusky *et al.*, 1988). Methylmercury exposure during development can also enhance the sensitivity of behaving animals to dopamine agonists (Cagiano *et al.*, 1990; Hughes and Sparber, 1978; Rasmussen and Newland, *in press*). Taken together, these hypotheses imply that the methylmercury and the PCBs could interact. A recent report, using striatal punches, indicated that an Aroclor 1254/1260 mixture and methylmercury interact synergistically to enhance dopamine concentrations *in vitro* (Bemis and Seegal, 1999). Structure-activity relationships and effects on whole animals have yet to be reported.

The animal studies suggest that one way of distinguishing among the compounds might be found in the different behavioral tasks used to evaluate learning and memory. While it is far from proved, there is some indication of a functional distinction between tasks that stress the acquisition of a response-consequence relationship and those that stress contextual control over behavior. Examples of the former would be found in the concurrent schedules in transition (Newland and Reile, 1999b). Examples of the latter would be found in tests of memory or in certain discrimination reversals. It is not

yet clear how this distinction would appear in human testing. Unfortunately, much of the human testing literature has evolved with little direct contact with the literature on animal behavior. Approaches that link more directly studies of human and nonhuman behavior hold some promise, however (Bickel *et al.*, 1991; Lattal and Perone, 1998; Paule *et al.*, 1999; Paule *et al.*, 1990).

The effects reviewed here have implications for human studies. Whenever there is a possibility of human exposures to both methylmercury and PCBs, it is likely that the PCBs will be in a mixture of congeners, and the specific congeners present will be affected by weathering and biomagnification. The presence of methylmercury and PCBs in the same food sources raises the possibility of interactions. Non-ortho-substituted PCBs have effect profiles that appear to resemble those seen with methylmercury. The presence of ortho-substituted PCBs may amplify the actions of methylmercury, and *vice versa*. Thus, it could be difficult to distinguish effects of methylmercury from those of the PCBs, and both classes of congeners contribute to this difficulty. If there is an endpoint that shows any promise at separating the two, it might be higher-order sensory testing, such as contrast sensitivity assessment. Unfortunately, there are no data on PCBs or on mercury-PCB interactions pertaining to this function.

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