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Full Length Article

Upper respiratory tract nociceptor stimulation and stress response following acute and repeated Cyfluthrin inhalation in normal and pregnant rats: Physiological rat-specific adaptions can easily be misunderstood as adversities

Juergen Pauluhn[a,b,c,*]

[a] Hannover Medical School, Hanover, Germany
[b] 4th Military Medical University, Xi'an, China
[c] Bayer Pharma AG, Department of Toxicology, Wuppertal, Germany[1]

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ABSTRACT

This paper reviews the results from past regulatory and mechanistic inhalation studies in rats with the type II pyrethroid Cyfluthrin. Apart from many chemical irritants, Cyfluthrin was shown to be a neuroexcitatory agent without any inherent tissue-destructive or irritant property. Thus, any Cyfluthrin-induced neuroexcitatory afferent sensory stimulus from peripheral nociceptors in the upper respiratory tract is likely to be perceived as a transient stimulus triggering annoyance and/or avoidance by both rats and humans. However, while thermolabile rats respond to such stresses reflexively, homeothermic humans appear to respond psychologically. With this focus in mind, past inhalation studies in rats and human volunteers were reevaluated and assessed to identify common denominators to such neuroexcitatory stimuli upon inhalation exposure. This analysis supports the conclusion that the adaptive physiological response occurring in rats secondary to such chemosensory stimuli requires inhalation exposures above the chemosensory threshold. Rats, a species known to undergo adaptively a hibernation-like physiological state upon environmental stresses, experienced reflexively-induced bradypnea, bradycardia, hypothermia, and changes in acid-base status during inhalation exposure. After cessation of the sensory stimulus, rapid recovery occurred. Physiological data of male and female rats from a 4-week repeated inhalation study (exposure 6-h/day, 5-times/week) were used to select concentration for a 10-day developmental inhalation toxicity study in pregnant rats. Maternal hypothermia and hypoventilation were identified as likely cause of fetal and placental growth retardations because of a maternal adaptation-driven reduced feto-placental transfer of oxygen. In summary, maternal reflex-hypothermia, reduced cardiac output and placental perfusion, and disruption of the gestation-related hyperventilation are believed to be the maternally mediated causes for developmental impairments. Thus, inhaled chemosensory substances may appear to be more toxic in rats than they will be in humans because the thermoregulatory response of rats to such stimuli can cause profound physiological adaptions that can easily be misunderstood as adversities in conventional inhalation studies in small rodents. The afferent threshold triggering such outcomes in rodents translate to perceptions of annoyance in humans. Consequently, hazard characterization and human risk assessment need to be focused on the chemosensory threshold rather than endpoints occurring downstream to rodent-specific homeostasis.

1. Introduction

Pyrethroids exert their toxicological effect through at least two distinct modes of action leading to their segregation into the Type I (T-syndrome), and Type II (CS syndrome) classes (Breckenridge et al., 2009). The α-cyano pyrethroid Cyfluthrin addressed in this paper belongs to the Type II class. Pyrethroids are axonic excitoxins and toxic effects are mediated through preventing the closure of the voltage-gated sodium channels in the axonal membranes. In general, voltage-gated ion channels are the predominant site(s) but transmitter-gated ion channels may also play a role in some clinical signs (Breckenridge et al., 2009; Choi and Soderlund, 2006; Gammon et al., 2012). Sub-

* Corresponding author at: Bayer AG, Crop Science Division, 40789 Monheim, Germany.
E-mail addresses: juergen.pauluhn@iCLOUD.com, juergen-pauluhn@t-online.de.
[1] Retired.

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channels are expressed exclusively in the peripheral nervous system, most notably in the sensory neurons, the trigeminal nerve and ganglia and are thought to mediate pain reception (Goldin, 1999). It was postulated that the Nav1.8 channel may mediate paresthesia that has been reported following pyrethroid contact especially with facial skin. While it is likely that paresthesia occurs following dermal contact to both Type I and Type II pyrethroids, anecdotally it has been reported to occur more intensely, at lower doses and to last longer for Type II pyrethroids than for Type I pyrethroids (Breckenridge et al., 2009). While inexperienced subjects may show signs of anxiety and distress, records from occupational exposures predominantly report signs of transient 'cold burning' of the directly exposed skin in the absence of phenotypes suggestive of any 'inflammatory irritation' (MAK, 2003; Wilks and Wilks, 2000). Animal models have been developed to profile the neurogenic, sensory irritation effect of pyrethroids following inhalation (Pauluhn and Machemer, 1998) or dermal administration (Cagen et al., 1984; McKillop et al., 1987). Data from such animal models could be compared to more limited results from human studies (Flannigan et al., 1985; Wilks and Wilks, 2000). The importance of the distinction of homeostatic, reflex-related effects through stimulation of peripheral nociceptors by heat/cold, haptic challenges, and/or chemesthesis is described in detail elsewhere (Brüning et al., 2014).

The focus of this review is to reanalyze data from past rat inhalation bioassays paying particular attention toward physiological endpoints that could serve as surrogates for comparisons of the pyrethroid-induced facial paresthesia perceived by humans and the rat-specific nociceptive physiological counterpart. This neurogenic afferent event originates from the sensory (nociceptive) neurons of the peripheral nervous system thought to mediate nociceptive pain reception in humans. This calls for a full understanding of the nociceptive physiology of the rat to sensation-related pain, triggering homeostatic adaptation and/or escape. In rats, a hibernation-like state can then be produced, principally by attenuating thermogenesis (Jinka et al., 2015). Thus, rats may be able to adapt to environmental changes in their natural habitat by reducing their energetic needs through the use of torpor, a reversible state of suppressed metabolic demand and decreased body temperature. One unifying observation in rats from pyrethroids causing paresthesia was a lower body temperature (Breckenridge et al., 2009). Under such conditions, the delivery of oxygen (O_2) to the tissues (cardiac output, perfusion pressure, hemoglobin concentrations, and oxygen saturation) is reduced. This is achieved by an autonomic and behaviorally regulated hypothermia to be aimed at diminishing the rats' physiologic requirements as a short-term strategy to match stressful conditions (Gordon, 2005; Jinka et al., 2015). The complex concatenation of secondary events to hypothermia calls for an introductory, concise excursion on thermoregulation to rationalize the course taken.

Under hypothermic conditions, blood gases and acid-base values are affected because of the increased solubility of gases at lower temperatures and reduced metabolic rate. As a rule, with a drop in core temperature, blood pH increases. $PaCO_2$ falls because of increased dissolved CO_2 and decreased CO_2 production. In spontaneously breathing rats exposed to Cyfluthrin aerosol, trigeminal nociceptive reflexes from the upper airways are afferently stimulated and lead to a forced decrease in minute ventilation by reflex-bradypnea. As a compensatory response, rats are then expected to show a respiratory alkalosis in acute studies and additionally an acidified urine in repeated inhalation exposure studies. Likewise, the oxyhemoglobin dissociation curve is shifted to the left at lower body temperature, resulting in a higher affinity of hemoglobin for oxygen. The resulting decrease in oxygen release to tissues is counterbalanced by decreased tissue oxygen demand at lower temperatures. When this occurs in pregnant rats, the entire feto-placental-maternal exchange of O_2 is affected and anoxia-related developmental deficits and/or delays can be expected. The impact of changes in thermoregulations on toxicological endpoints is dealt with in detail elsewhere (Gordon, 1991, 1993, 2005; Gordon et al., 2007; Rhoades, 2013; Székely, 2000; Tanner, 2013; Whalan et al., 2015; Watkinson and Gordon, 1993; Watkinson et al., 2001; Witzmann, 2013; Wolansky and Tornero-Velez, 2013; Zanelli et al., 2011).

Induced perturbations to any one of the involved components to maintain gestation could easily upset the balance of the entire maternal-feto-placental system (Carney, 1996; Carney et al., 2011). Thus, it should not be surprising that maternal physiological disruptions have adverse developmental consequences. The most salient physiological changes placed on the dam to serve the needs of the growing conceptuses mandate gestation-related increase in respiratory minute ventilation (MV) to assure stability of maternal blood gases – oxygen and carbon dioxide (Omo-Aghoja, 2014). One would expect an increased MV to increase the inhaled pyrethroid dose (Leavens et al., 2006), but this does not happen at concentrations causing upper respiratory tract nociceptor stimulation triggering maternal reflex-bradypnea, hypoventilation, and hypothermia. While these reflexes protect the dam, they can harm her growing fetuses. Fetal hypoxia can occur when maternal oxygenation is compromised, maternal perfusion of the placenta is reduced, or delivery of oxygenated blood from the placenta to the fetus is impeded. Maternal-fetal acid-base derangements in pregnancy are reported as important factors causing derangements on fetal outcome (Omo-Aghoja, 2014). Acid–base disturbances in hypothermia are complex (Rhoades, 2013; Tanner, 2013). Respiration and cardiac output typically are depressed more than metabolic rate, and a mixed respiratory alkalosis and metabolic acidosis results because of CO_2 retention and the associated shift of the hemoglobin-O_2 dissociation curve to the left. Under equilibrated conditions, hypothermia parallels decreased metabolism with decreased $paCO_2$ which then leads to a state of compensated respiratory alkalosis. The fetal oxyhemoglobin dissociation curve is left-shifted in comparison with the dams' because fetal hemoglobin has a lower binding affinity for 2,3-diphosphoglycerate. Overlapping affinity may occur when hypothermia shifts the maternal oxyhemoglobin dissociation curve further to the left, resulting in a higher affinity of maternal hemoglobin for oxygen with decreased supply of oxygen to tissues and the placenta (Zanelli et al., 2011). Both of these mechanisms shift the hemoglobin oxygen dissociation curve opposite to the direct pH (Bohr) effect. Thus, factors that normally facilitate oxygen delivery across the placenta may be disrupted under conditions of hypothermia and deranged acid-base status (Bellingham et al., 1971; Dash et al., 2016; Dunlop, 1999; Kofstad, 1996; Weber and Campbell, 2011).

The objectives of this review of past studies with Cyfluthrin are to retrospectively search for common denominators of Cyfluthrin-induced chemosensations in rats and humans. Opposed to chemicals typically evoking sensory and inflammatory irritation at low and high exposure levels, respectively, this neuroexcitatory pyrethroid can be judged to trigger afferent portal-of-entry related chemosensations (nociception) in the absence of any irritation-related adversities. Emphasis is directed to identify stress-related biomarkers of effects and how they coincide with reflex-induced changes in breathing patterns, cardiopulmonary function, and body temperature. An ancillary focus of this review is to compare the most salient subjective and objective findings observed in volunteers exposed to Cyfluthrin-aerosol discharged from a spray-can with those from Cyfluthrin-aerosol exposed rats. Objective scoring of the human equivalent of this type of *paresthesia* is clinically challenging because of the subjectivity of '*perceived irritation*' and the associated psychological response (Verberk, 1977). This paper tests the hypothesis that rats and humans have similar characteristics of afferent nociception by stimulation of the nociceptors responsive to Cyfluthrin. This comparison considered data from acute, repeated standard and specialized developmental inhalation exposure studies in rats and those from a previously published study in human volunteers (Pauluhn and Machemer, 1998).