Expert Opinion

Cyfluthrin-Induced Sensory Irritation in Rats and its Human Relevance

by

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3 EXECUTIVE SUMMARY

Pyrethroids are axonic excitoxins, the toxic effects of which are mediated through preventing the closure of the voltage-gated sodium channels in the axonal membranes. When the toxin keeps the channels in their open state, the nerves cannot repolarize, leaving the axonal membrane permanently depolarized, thereby paralyzing the organism. Type II pyrethroids, such as Cyfluthrin, can exert their neuroexcitatory Mode of Action (MoA) upon direct exposure of the skin. Due to the inverse relationship of neuroexcitation and temperature, sensitivity increases at skin locations with lower than normal body temperature, e.g., facial skin. The most instant warning signal upon exposure to pyrethroids-aerosols is *paresthesia*, which is a sensation of tingling, tickling, pricking, or burning of a person's skin with no apparent and obvious long-term physical effect. In

accidentally exposed humans the manifestation of a Type II pyrethroid-induced paresthesia was shown to be acute and transient. Notably, such local events are hardly modulated by kinetic or dispositional factors (apart from the fact that some individuals may show hypersusceptibility to sodium channel blockers). Objective scoring of this type of *paresthesia* is clinically challenging because of the subjectivity of '*perceived irritation*' (non-adverse nociceptive sensation) in the absence of any phenotypes of any '*inflammatory irritation*' (adverse outcome).

This particular local MoA is not expected to occur even at high-dose oral studies. Conversely, following inhalation, such sensation may already be stimulated upon exposure of the facial skin, eyes and/or nares. Typical for any neuroexcitation, alike exposure to cold or heat, afferent *nociceptive* stimulation occurs concentration-dependently, like a wash-in/wash-out event. Conversely, injury because of '*inflammatory irritation*', always is a concentration x time (Cxt)-proportional event with carry-over of adversity from one exposure to the next.

Numerous regulatory-driven and MoA-based inhalation studies on rats were executed to verify / refute the hypothesis that the early events observed in rats are related to a typical rat-specific translation of generic nociception. Suffice it to say, even if the *afferent* nociception between rats and humans is not assumed to be appreciably different, its *efferent* translocation to 'successful escape' should be rat-specific. For the rat this translates to escape into any type of burrow. There the rat may have to negotiate a hostile environment, e.g., low levels of oxygen, high levels of CO_2 or gases produced by putridity. The rats' nociceptive system is designed to promptly adjust to this type of hostile environment with dramatic changes in metabolisms by reflex-bradypnea and hypothermia. To the contrary, the *efferents* of humans take actions for flight and/or fight. Accordingly, there is no evolutionary need for humans to decrease body temperature. Additionally, the ≈250-fold higher body weight of humans relative to rats cause an inertia of temperature that may preclude hypothermia to occur. As known from controlled volunteer studies, any type of paraesthesia-like chemosensation may be perceived as anxiety or nuisance, depending on previous experience.

To summarize, the overwhelming experimental evidence from rat and human studies demonstrate unequivocally that the effect thresholds are acute in nature, without evidence of any cumulative or aggravating outcomes following recurrent exposures. Hence, caution is advised to translate the physiological rodent-specific nociceptive response to humans. Accordingly, although evidence of '*perceived irritation*' was obtained, '*inflammatory irritation*' did not occur in any study. Therefore, the observed responses do not fulfil the criterion for being a respiratory tract irritant.

4 INTRODUCTION

Many physiological features of the control of breathing in humans and rats are similar with regard to the 'afferent sensing' of external stimuli. However, these afferents must be translated into human- and rat-specific afferents to end-up in a meaningful physiological response. Differences are necessary due to the fact the rats breathe much faster than humans with airway lumens much smaller than humans. Hence, the sensory perception (afferent & neurogenic)) leads to differing nociceptive responses. In human airways, abundant seromucous glands (under neurogenic control) are found to increase the protective layer to irritant stimuli. A similar response in rats would plug the airway resulting in a totally compromised ventilation. Therefore, rodent nociception is aimed at to reduce instantly the inhalation of any substance triggering neurogenic sensation by upper airway trigeminal-stimulation driven 'reflex-bradypnea' (Pauluhn, 1999; Pauluhn et al., 1996, Whalan et al., 2015).

As conceptualized in Fig. 1, the rat has two pathways of heat dissipation, skin and exhaled breath. At that moment 'reflex-bradypnea' is triggered, the heat-dissipation via exhaled air is blocked and oxygen supply is decreased. This reflex is operative also on the hart to maintain the physiological required relationship of 4 heat beats per 1 breath. Due to the thermal 'inertia' of the body depression of respiration precedes hypothermia (the example given was from a study with Cyfluthrin on rats with implanted radio transmitters). Typical for a reflexively-induced locally-triggered response is the rapid onset and recovery after cessation of exposure to the stimuli. Suffice it to say, hypothermia goes along with reduction of oxygen consumption, metabolism, and CO_2 production. Recalling the Henderson-Hasselbalch-equation pH = pK + log {[HCO_3^-]/[H_2CO_3]} adjusted to CO_2 becomes pH = 6.1 + log {[HCO_3^-]/[CO_2]}, any decrease in the partial pressure of CO_2 should be phenotypically manifested as respiratory alkalosis (*vide infra*).

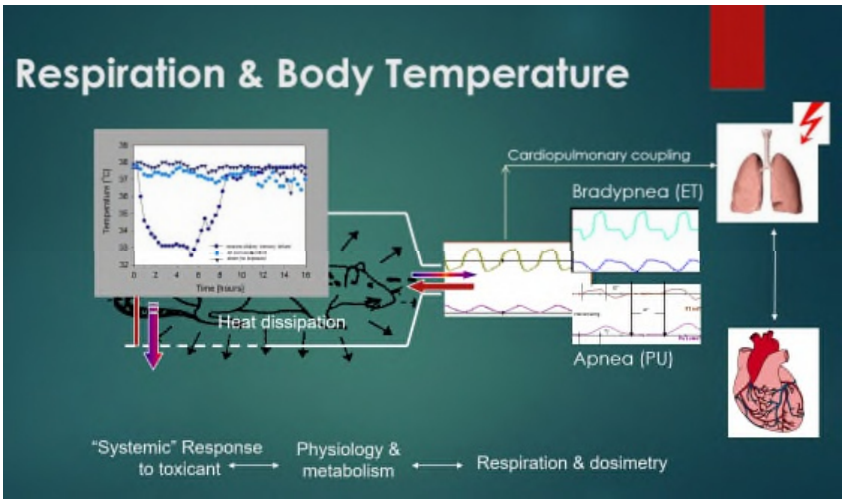

Figure 1: Interrelationship of hypothermia and sensory irritation-induced reflex bradypnea.

Cyfluthrin, as a Type II pyrethroid, is known to be a neuroexcitatory stimulant. One may argue that the observed outcome may easily confounded with central neurostimulation. However, this may readily be identified by the differing breathing patterns shown in Fig. 1. Also the time course of onset and recovery are expected to be different. Nonetheless, this interrelationship is also given for a direct acting respiratory tract irritant with no systemic bioavailability (Fig. 2). The evidence provided in Fig. 2 demonstrates an unequivocal concordance between local sensory irritation and nociception-related hypothermia. Notably, following repeated inhalation studies of rats to Cyfluthrin aerosol there was no functional or histopathological evidence of any adverse respiratory irritation and/or inflammation. The degree of Cyfluthrin-induced reflex-bradypnea was not affected in repeated exposure subchronic inhalation studies. This means, opposite to inflammatory irritants, recurrent exposure of rats to Cyfluthrin aerosol did not affect the responsiveness of the nociceptive system. In summary, evidence of any Cyfluthrin-induced RTI-related adversity was absent. Conversely, for phenyl isocyanate a progressive irritation/inflammation-related deterioration of the respiratory tract occurred (for details see Pauluhn at al., 1995).

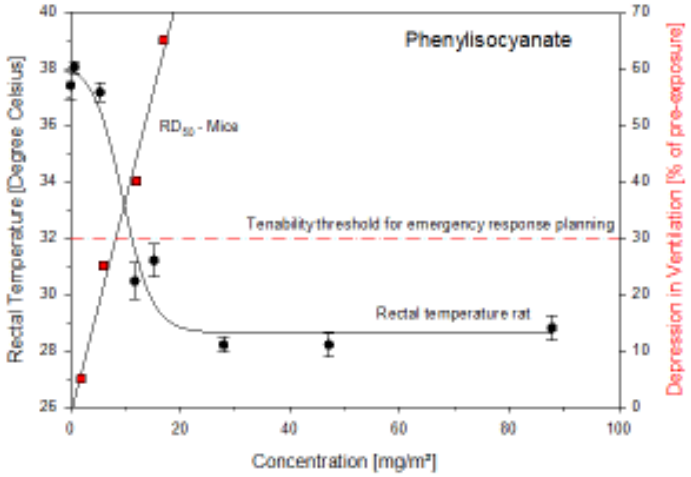

Figure 2: Interrelationship of hypothermia (rats) and sensory irritation-induced reflex bradypnea (mice) following inhalation exposure to phenyl isocyanate vapor. The RD_{50} of rats and mice was essentially identical (for details see Pauluhn at al., 1995)

Reflex-bradypnea associated hypothermia with decreased oxygen consumption has major impact on multiple physiological and biochemical reactions. Body temperature affects the solubility of gases and also shifts the oxygen-hemoglobin dissociation curve hindering the dissociation of oxygen from haemoglobin to tissue. Blood gases are measured in machines where the electrodes are usually held at 37°C and provide the option for these primary measurements to be automatically "corrected" to the patient's actual body temperature. Controversy has surrounded the use of "temperature-corrected" blood gas results, so that there has been lack of consistency in their adoption (in patients). This issue may be complicated further as the used machinery used was adopted to the blood of humans rather than rats. Nonetheless, the time-course of elaborating a typical state of hypothermia coincided with decreased arterial pCO_2 and increased arterial blood pH (*quod erat demonstrandum*; see also Gordon et al., 2007).

This observation is also entirely consistent with the observations obtained from hypothermia rat models (Alfaro and Palacios, 1985): Interestingly, the interactions between components that contribute to acid-base homeostasis were studied in the first steps of acute hypothermia [body temperature (Tb) 37-31 °C] in awake unrestrained rats as an experimental model of accidental hypothermia in mammals. The concurrent changes in blood gases, plasma ions, and plasma protein concentrations in arterial blood were analyzed. Acute decreases in Tb decreased pCO_2 and increased pH. It is concluded that, during the first stages of body cooling, the blood acid-base status of conscious hypothermic rats is affected by pCO_2 changes, apparently because of uncoupled changes between ventilation and metabolism, but it is also affected by a transitory metabolic disorder due to ion imbalance. This equivalence provides an unequivocal relationship of hypothermia and changes in arterial acid-base status.

No doubt, Cyfluthrin-exposed rats elaborated early signs suggestive of sensory irritation and associated changes in general behavior throughout all inhalation studies. The similarity of NOAELs from single to repeated inhalation studies support the conclusion that this threshold is non-cumulative and contingent on local 'sensations and nociception' which cannot equaled with that type of adversity occurring at higher exposure intensities.

5 TRANSLATIONAL TOXICITY

As already alluded to above, *afferents perception* was assumed somewhat identical between human and rats. However, marked species differences with regard to nociceptive *efferent* responses were expected to occur (see Executive Summary). A 'proof-of-principle' human volunteer study was executed to better understand the equivalence of the afferent susceptibility of humans relative to rats (Pauluhn and Machemer, 1998). The objective of study was to reveal whether humans are more, less or of equal susceptibility. It was realized at the outset of study that 'paraesthesias' have no real objective component; however, may be associated with some transient vasodilation of nasal capillaries or eye reflexes. Volunteers were informed as to how 'paraesthesias' may feel and the observing clinicians interrogated all exposed subjects repetitively during the course of exposure. The exposure as such was quasistatic with all volunteers in one room. The records made from one volunteer could have influenced the remaining volunteers. The study was not designed to derive any quantitative data in terms of human concentration-response-relationships. This would have required a double blind placebo control study with dynamic aerosol supply. Nonetheless, the objective of study was achieved. The most sensitive endpoint recorded was related to upper respiratory tract (sensory) irritation in the range of the same sensory irritant threshold concentration already established in rats. This confirmed the starting hypothesis, that the physiological *afferent* portal-of-entry effects observed in rats and humans are identical and do not require any further interspecies adjustment factors. Likewise, quantitative-risk assessment should solely rely upon rat inhalation studies due to the much better control of exposure atmospheres and guideline-compliant nature of studies.

The weakness of human studies has been exemplified with ammonia by a study from Verbek (1977) which aptly shows the influence of conditioned fear in study cohorts "experienced toxicology experts" and unconditioned fear in the cohort of students not familiar with ammonia (or toxicology at all). None of the psychophysical-psychological effects reported by this author in these ammonia-exposed cohorts were really progressive with exposure duration. No negative or odor-related benchmark substance was used in this non-blinded study nor was there any robust explanation as to how the categorization of 1/2 = just/distinctly perceptible, 3 = nuisance, 4 = offensive, and 5 = unbearable were defined for objective/subjective observational endpoints. In neither cohort the nuisance score was exceed for eye irritation. In the "conditioned expert cohort" the throat score never exceeded the 'perceptible' range while the cough score was below 1 at all examination time points. For the eye effects evidence of adaptation existed, a finding typical for sensory phenomena. Despite the possibility of nasopharyngeal-bronchial reflex loops, no reflexively-related changes in lung function following histamine bronchoprovocation challenge were noticed up to the maximum concentration of 140 ppm NH_3 examined over an exposure period of 2 hours. The author attributed the differences in susceptibility of the two cohorts to uncontrollable psychological factors. Collectively, the Verbek study seems to demonstrate exactly the same problems related to "perception" as observed in the volunteer study with Cyfluthrin.

6 REGULATORY SIGNIFICANCE

To summarize, reflex bradypnea (RB) is a protective sensory reflex that allows rodents - but not humans - to markedly reduce their exposure to inhaled upper respiratory tract (URT) sensory irritants such pyrethroids. When a sensory irritant exposure above some biological threshold stimulates trigeminal nerves in the upper respiratory tract (URT) or facial skin, rodents experience a rapid and sustained decrease in ventilation (as much as 90%) so they inhale a much lower chemical dose than if they were breathing normally. This bradypnea is typically accompanied by decreases in body temperature, metabolic rate, heart rate, and activity, and altered acid-base

status. These protective physiological effects may be misconstrued as adverse “systemic” outcomes. This evidence demonstrates that behavioral and developmental effects due to RB may not be relevant to humans. RB in pregnant dams can result in fetal hypothermia, impaired placental transfer of O_2 (hypoxia) and CO_2 (hypercapnia), developmental disturbances, and other effects that may be erroneously attributed to a test article. The impact RB can have on human health risk assessments has not received the attention it deserves from toxicologists and risk assessors, largely because current testing guidelines do not require examination of RB-related endpoints. This analysis shows the major impact RB can have on the interpretation of findings, and it demonstrates why it may be necessary to adjust points-of-departure (PODs) in risk assessments of inhaled irritants to make them health protective for humans.

With regard to regulatory impact, caution is advised not to translate any physiological rodent-specific nociceptive response related to ‘*perception*’ to humans. Accordingly, evidence of ‘*perceived irritation*’ should not be equalled to ‘*inflammatory irritation*’ that did not occur in any study. Therefore, the observed responses do not fulfil the criterion for being a respiratory tract irritant in either rats or humans.

7 REFERENCES

Alfaro V, Palacios L (1985). Acute mild hypothermia in awake unrestrained rats induces a mixed acid-base disorder.J Appl Physiol. 1996 Jun;80(6):2143-50.

Gordon, C.J., Spencer, P.J., Hotchkiss J, Miller, D.B., Hinderliter, P.M., and Pauluhn, J. (2007). Thermoregulation and its influence on toxicity assessment. Toxicology 244, 87-97.

John E. Whalan[1], Jűrgen Pauluhn[2], Andrew D. Kraft[1], and Susan Makris[1], 2015. The Impact of Rodent Reflex Bradypnea on Human Health Risk Assessments of Inhaled Irritants[1]U.S. EPA, National Center for Environmental Assessment, [2]Bayer Pharma AG-Toxicology (retired), Wuppertal, Germany. Poster at the Annual Meeting of the US Society of Toxicology, San Siego, March 2015.

Pauluhn, J. (1999). Hazard identification and risk assessment of pyrethroids in the indoor environment. *Toxicology Letters* 107:193-199.

Pauluhn, J. and Machemer, L.H. (1998). Assessment of pyrethroid-induced paraesthesias: comparison of animal model and human data. *Toxicology Letters* 96-97: 361-368.

Pauluhn, J., Rüngeler, W., and Mohr, U. (1995). Phenylisocyanate Induced Asthma in Rats Following a Two-Week Exposure Period. *Fundam. Appl. Toxicol.* 24:217-228.

Pauluhn, J., Steffens, W., Haas, J., Machemer, L., Miksche, L.K., Neuhauser, H., and Schüle, S.(1996). Toxikologische Bewertung von Pyrethroiden in Innenräumen: Aufgezeigt am Beispiel Cyfluthrin und Permethrin. *Gesundheitswesen* 58: 551-556.

Verberk, M.M. 1977. Effects of ammonia in volunteers. *Int. Arch. Occup. Environ. Hlth.* 39, 73-81.