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Repeated inhalation exposure of rats to an anionic high molecular weight polymer aerosol: Application of prediction models to better understand pulmonary effects and modes of action

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ABSTRACT

Opposed to the wealth of information available for kinetic lung overload-related effects of poorly-soluble, low-toxicity particles (PSP), only limited information is available on biodegradable high molecular weight (HMW) organic polymers (molecular weight >20,000 Da). It is hypothesized that such types of polymers may exert a somewhat similar volume displacement-related mode of action in alveolar macrophages as PSP; however, with a differing biokinetics of the material retained in the lung. This polyurethane polymer was examined in single and 2-/13-week repeated exposure rat inhalation bioassays. The design of studies was adapted to that commonly applied for PSP. Rats were nose-only exposed for 6 h/day for the respective study duration, followed by 1-, 2- and 4-week postexposure periods in the single, 2- and 13-week studies, respectively. While the findings in bronchoalveolar lavage (BAL) and histopathology were consistent with those typical of PSP, they appear to be superimposed by pulmonary phospholipidosis and a much faster reversibility of pulmonary inflammation. Kinetic modeling designed to estimate the accumulated lung burden of biopersistent PSP was also suitable to simulate the overload-dependent outcomes of this biodegradable polymer as long as the faster than normal elimination kinetics was observed and an additional 'void space volume' was added to adjust for the phagocytosed additional fraction of pulmonary phospholipids. The changes observed following repeated inhalation exposure appear to be consistent with a retention-related etiopathology (kinetic overload). In summary, this study did not reveal evidence of any polymer-specific pulmonary irritation or parenchymal injury. Taking all findings into account, 7 mg polymer/m^3 (exposure 6 h/day, 5-days/week on 13 consecutive weeks) constitutes the point of departure for lower respiratory tract findings that represent a transitional state from effects attributable to an overload-dependent pulmonary inflammation and phospholipidosis. In regard to extrapulmonary toxicity, no effects were found up to the maximum concentration of 107 mg/m^3 examined.

1. Introduction

The objective of this paper was to analyze whether the examined slowly biodegradable poorly soluble high molecular weight (HMW) anionic polyurethane-polyurea macromolecule (MW >20,000 Da) has any toxicological properties in common with biopersistent poorly soluble low-toxicity particles (PSP). The polymer structure contains both hydrophilic and hydrophobic segments, which impart a unique and coexisting combination of hydrophilic and hydrophobic properties. This colloidal system can be dispersed in water and may function as an elastic film former on both hydrophilic and hydrophobic interfaces. This poorly soluble polymer may share, at least in part, several characteristics with PSP when inhaled, for instance, it may interact with the amphiphilic surfactant by forming complexes with phospholipids. Either as an inhaled polymer or an endogenously formed complex any recurrent long-term inhalation exposure may putatively result in an overload-like etiopathology within the lung; however, these structures are likely to be cleared faster than typical PSP. Film-forming per se can potentially be causing detrimental conditions at the alveolar blood–air barrier as was shown for sprays containing surface-active materials (Nørgaard et al., 2013; Pauluhn et al., 2008). Apart from aspects of occupational and consumer safety, also regulatory needs have been articulated to better characterize this category of HMW organic polymers. US-EPA has reasoned a concern for potential fibrosis of the lung or other pulmonary effects that may be caused by

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long-term inhalation of respirable particles with HMW polymers (http://www.epa.gov/oppt/newchems/pubs/hmwtpoly.html).

Therefore, two major modes of action (MoA) can be expected: (i) a direct pulmonary toxicity of polymer directly at the initial site of alveolar deposition due to the physicochemical properties of this polymer. The phenotype and time-course of the expected effects are likely somewhat similar to the 'acute' effects of PSP. In context with PSP, these types of effects are commonly alleged to be associated with particle surface area/activity. (ii) Upon recurrent exposures, unspecific inflammation proportional to the cumulative lung burden of retained polymer may occur as result of kinetic lung overload. However, the cumulative yet homeostatic displacement volume of the polymer within alveolar phagocytes may already be exceeded at lower polymer levels due to the additional uptake of phospholipids within the same compartment. To disentangle these putative MoAs, rats were exposed nose-only to the aerosolized aqueous colloidal dispersion of the polymer for 1×6 h, 2- and 13-weeks (6 h/day on five consecutive days/week) followed by 1-, 2-, and 4-week postexposure periods, respectively. These studies were designed to test the hypothesis detailed above, namely whether the polyurethane-polyurea polymer-induced pulmonary changes are consistent with any kinetic overload-like phenomenon following repeated inhalation exposure as observed and modeled in studies on rats with biopersistent PSP (Pauluhn, 2011). Kinetic modeling was applied to better understand and predict the anticipated pulmonary kinetics of the inhaled polymer-aerosol and associated phospholipidotic effects. This mechanistic understanding is considered mandatory for the derivation of safe occupational and consumer exposure levels.

2. Methods

2.1. Test material

Linear anionic hexamethylene diisocyanate monomer-based polyurethane-polyurea HMW polymer of >20,000 Da. When dispersed in water, the insoluble content of dispersion was approximately 30%.

2.1.1. Animals

Specific-pathogen-free, healthy young adult male and female SPF bred Wistar rats, strain Hsd Cpb:WU (SPF), from the experimental animal breeder Harlan-Nederland (NL), AD Horst, were used. At the commencement of the study the rats were 2 months old and were acclimatized for 1 week (single exposure) or 2 weeks (repeated exposure) prior to the beginning of study. Animals of the 13-week study were identified by individual transponders (IMI 1000, Biomedic Data Systems, BMDS), animal color marks, and cage-labels. The transponders were subcutaneously (flank) injected after randomization using the IMI injection equipment. On each exposure day the correct animal location in the respective chamber was verified using a digital reader (DAS 5002, Biomedic Data Systems). Rats were singly housed in polycarbonate cages, containing low-dust wood shavings as bedding material. Ration consisted of a standard fixed-formula diet (ssniff® R/M-H pellets maintenance diet for rats and mice; ssniff Spezialdiäten GmbH, http://www.ssniff.de) and tap water (drinking bottles). Both food and water were available ad libitum. Housing conditions were in accordance to the EU animal welfare regulation. Animal holding rooms had a dark/light cycle12 h/12 h; artificial light from 6.00 a.m. to 6.00 p.m. Central European time. Temperature and relative humidity of animal holding rooms were in the range of 22 °C and 40–60%, respectively.

2.2. Study rationale and study design

The studies were performed in an animal care-approved laboratory in accordance with the German Animal Welfare Act and European Council Directive 2010/63/EU (EEC, 2010). All procedures observed the procedures called for by OECD TG#403 (2009a)(acute study) and OECD TG#413 (2009b) with particular emphasis on OECD GD#39. This study was conducted in compliance with the OECD Principles of Good Laboratory Practice as revised in 1997 (ENV/MC/CHEM(98)17) and with the revised German Principles of Good Laboratory Practice according to Annex I German Chemicals Act (Bulletin of the revised form of the chemicals act of 13th July, 2013 (BGBI.I pp. 2565), Federal Law Gazette Volume 2013 (Part I No. 55, section 6, §19 (all subparagraphs included), issued at Bonn 6th September, 2013).

In the 13-week repeated exposure study actual concentrations of 5, 26, and 107 mg/m^3 were used. Based on the outcome of sighting pilot inhalation studies, respiratory tract effects that may cause undue irritant stress animals or irreversible effects could be excluded at the outset of study. Based on the findings of the 2-week repeated exposure pilot study, 5 mg/m^3 was expected to be the NOAEL of study because the PMN recruitment in bronchoalveolar lavage observed at the intermediate concentration of 26 mg/m^3 was still in a range considered to be non-adverse. Thus, this concentration was likely to be in the range where overload-like local effects may start to occur. The high-dose group was expected to show unequivocal evidence of lung overload.

2.2.1. Pilot studies

Previous considerations on PSP-related mechanisms support that two modes of action (MoA) have to be accounted for to better qualify the principal MoA of PSP (Pauluhn, 2011, 2014). For conventional PSP a deposition-dependent acute MoA_I as well as a retention-related chronic MoA_{II} was articulated. Consistent with the suggested procedures for evaluating these MoAs, the pre-studies were stratified as follows: (i) Single 1×4 h exposure inhalation study and 14-day postexposure observation period in rats. Mortality did not occur up the maximum technically attainable concentration of 910 mg/m^3 (MMAD 2.8 μm, GSD 2.5). At this concentration rats displayed transient signs suggestive of bradypnea and labored breathing patterns, including hypothermia. (ii) Single 1×6 h inhalation exposure of male rats followed by serial BAL-analyses on postexposure days 1, 3, and 7 with focus on MoA_I-related deposition-dependent outcomes. A similar protocol was utilized to categorize the toxic potency of pulmonary irritants (Pauluhn, 2004). (iii) The putative MoA_{II}-related retention-dependent profile was examined in a repeated 2-week repeated exposure inhalation study followed by a 2 week postexposure period. The applied methodologies were essentially similar to those used in the 13-week study (for details see below). In brief, this study (iv) examined the polymer at gravimetric filter concentrations of 0 (vehicle water), 5.4, 22.3 and 120.7 mg/m^3 (MMAD 0.9–1.4 μm, GSD 2.2–2.5). Additional rats (control and high-level exposure groups) were allowed to recover during a 2-week post-exposure period. The endpoints examined were similar to those of the 13-week study.

2.2.2. Subchronic 13-week study

At the end of the acclimatization period male and female rats were randomly assigned to four exposure groups of rats each of which was exposed by nose-only to actual gravimetric concentrations 0 (vehicle), 5, 26, and 107 mg/m^3 for 13 weeks (6 h/day, 5-times/week on 13 consecutive weeks) followed by a 4-week exposure-free recovery period. Clinical observations were systematically performed on individual rats before and after each exposure and on exposure-free weekends. The following reflexes