



Consequent stages of developing a multi-compartmental mechanistic model for chronically inhaled nanoparticles pulmonary retention



Boris A. Katsnelson*, Marina P. Sutunkova, Ludmila K. Konyshcheva, Svetlana N. Solovyeva, Ilzira A. Minigalieva, Vladimir B. Gurvich, Larissa I. Privalova

The Ekaterinburg Medical Research Center for Prophylaxis and Health Protection in Industrial Workers of the Rospotrebnadzor, Ekaterinburg, Russia

ARTICLE INFO

Keywords:

Nanoparticles
Lung retention
Multicompartmental models

ABSTRACT

The paper retraces the development of a mechanistic multicompartmental system model describing particle retention in lungs under chronic inhalation exposures. This model was first developed and experimentally tested for various conditions of exposure to polydisperse dusts of SiO₂ or TiO₂. Later on it was successfully used as a basis for analyzing patterns in the retention of nanoparticles having different chemical compositions (Fe₂O₃, SiO₂, NiO). This is the first publication presenting the outcomes of modeling lung retention of nickel oxide nano-aerosols under chronic inhalation exposure.

The most significant adaptation of the above-mentioned model to the conditions of exposure to metal-oxide nanoparticles is associated with the need to describe mathematically not only the physiological mechanisms of their elimination but also their solubilization “in vivo” bearing in mind that the relative contribution of the latter may be different for nanoparticles of different nature and predominant in some cases.

Using nickel oxide as an example, it is suggested as well that damage to the physiological pulmonary clearance mechanisms by particularly toxic nanoparticles may result in lung toxicokinetics becoming nonlinear.

1. Introduction

Simulating real-life exposures is one of the forefront challenges of the modern preventive toxicology. Within this widely discussed problem [1], however, one of the least developed (even if being long ago recognized) is the adequate experimental and mathematical simulation of long-term low-level inhalation exposures to particulates.

It should be mentioned, in this respect, that one of the oldest basic paradigms of general toxicology, the so-called dose-response principle [2] is usually regarded as virtually synonymous to the exposure-response relationship. However, in the situation of inhalation exposure this generalization of the said paradigm creates the most explicit uncertainty. In fact, in this situation the first challenge is to establish a correct dose-exposure dependency.

That is why mathematical prediction of inhaled particle *deposition* in the human respiratory system is of immediate practical significance for the dosimetry of toxic and radioactive aerosols. It has therefore been an important subject-matter for researchers, particularly in the mid-20th century. Studies in this area led to the development in the mid-1960s of the well-known and still relevant model of the International Commission on Radiological Protection [3]. A brief review of the

studies in this subject area with a particular focus on nanoparticle retention is available in Fröhlich and Salar-Behzadi article [4].

At the same time, it is obvious that long-term *retention* (i.e. accumulation) of particles in lungs may be directly measured in animal experiments only, and predicting such retention in animals based on one or another of the mathematical models would hardly be of interest from the practical point of view. However, systems analysis of the mechanisms controlling such retention, including their description by means of mathematical modeling, may be extrapolated to humans with due account not only for possible quantitative but also essential interspecies differences. Such modeling strengthens the theoretical basis of human health risk assessment and management and thus is highly important, even if indirectly, on the practical level.

It is well known that real-life environmental exposures, including those through particle inhalations, are mostly multifactorial [5,6]. To simulate such situations is an especially challenging task which, as far as inhalation exposures mathematical modeling goes, might be approached only on the basis of one-factor exposures models.

In this paper we will be dealing with system models rather than with data models. The latter would simply approximate actual experimental data by a mathematical function describing the relationship between

* Corresponding author.

E-mail address: bkaznelson@etel.ru (B.A. Katsnelson).

<https://doi.org/10.1016/j.toxrep.2019.03.008>

Received 26 February 2019; Received in revised form 23 March 2019; Accepted 26 March 2019

Available online 27 March 2019

2214-7500/ © 2019 Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

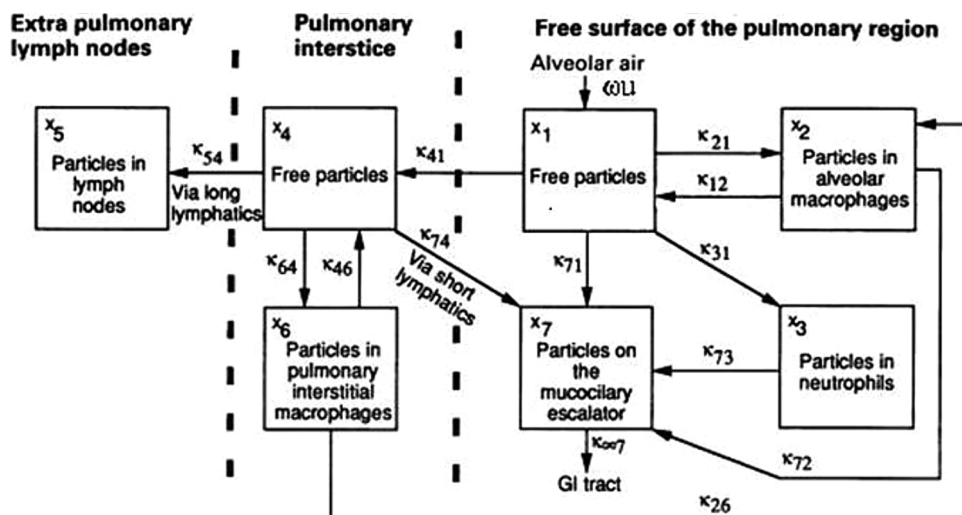


Fig. 1. Structure of the multicompartmental model for the kinetics of retention, redistribution and clearance of insoluble dust particles deposited in the pulmonary region of the rat lung [7]. Notation of constants is explained in the Subsection 2.2.

the exposure time and the mass of the retained particulate. On the contrary, the development of a system model begins with a simulation of the presumed structure and functioning of the system modeled followed by selection of its quantitative parameters such that would ensure a satisfactory fit of the model prediction (of the said mass, in our case) to respective experimental values. Since the first step of such modeling is based on certain theoretical premises, this correspondence may convey an important, albeit non-exhaustive confirmation that these premises were essentially correct. Indeed, the goal of system modeling is just obtaining such confirmation.

For our team, the starting point was a mathematical model for the retention of airborne polydisperse dust particles in tissues of lungs and lung-associated lymph nodes that we proposed a quarter of a century ago and tested repeatedly [7–9]. It should be noted that at about the same time, and independently of us, the so-called physiology-oriented multicompartmental kinetics model simulating pulmonary retention data on biopersistent, noncytotoxic aerosols in long-term inhalation exposures of rats was developed by one of the most prominent researchers in this field, late Werner Stöber [10], and then by the Edinburgh research team [11].

As shown in Fig. 1, in our case it was a multicompartmental model which describes the content of a substance in an organ as distributed among several non-isokinetic compartments partly correlated with certain morpho-functional structures while the flows of the substance between these compartments are presented as a system of linear differential equations. The experimental data (both our own and those available in the literature) and theoretical premises that determined this structure of the model were set out in detail in the above-mentioned first publications [7–9] to which we'd like to refer the readers of the present paper. In brief, the main postulates underlying this structure were as follows:

- 1 The phagocytosis of particles deposited on the free surface of the alveolar region prevents them from penetrating into the pulmonary interstitium and is likely to facilitate their transfer into the zone of the mucociliary "escalator".
- 2 Alongside the internalization of particles by alveolar macrophages (AM), the role of an auxiliary pulmonary clearance effector is performed by neutrophil leukocytes (NL), whose contribution is the greater, the higher the cytotoxicity of the particles¹.

- 3 Only particles that have not been internalized or were released again upon destruction of the macrophage that had engulfed it are capable of penetrating through the alveolar wall and further transport with the lymph (by short paths to the mucociliary region or by long paths into the systemic lymphatic stream to be then retained in lung-associated lymph nodes or to attain the bloodstream).
- 4 Given that mineral dusts then studied accounted for a rather low particle burden on a neutrophil, the destruction of these cells was assumed to be of little significance and thus was ignored by the model.
- 5 The interstitial macrophage pool is one of the widely recognized sources for the recruitment of alveolar macrophages, which accounts for the return into the alveoli of some particles that penetrated into the interstitium and were phagocytized by cells of this pool.

It is easy to notice that we did not then allow for the probable toxicokinetic role of particle solubilization *in vivo*, assuming it to be insignificant in terms of explicitly approximate modeling. In this respect, our approach to solubilization is not very much different from that of the authors referred to above [10,11]. On the other hand, not only did those authors disregard the toxicokinetic effects of damage to macrophages by particles engulfed by them but often emphasized that they were proposing a retention model for *non-cytotoxic* particles. On the contrary, our model assumed a certain degree of cytotoxicity to be an inherent property of any mineral particle and simulated real toxicokinetic effects associated with just this characteristic: for instance, the above-mentioned release of internalized particles assumed to be directly dependent on the degree of their cytotoxicity.

The model constants were first identified by the data of a chronic inhalation experiment with quartzite dust in a very high concentration (about 90 mg/m³) and then verified by the data of two other experiments with the same dust. The same model reliably predicted (by means of independently justified adjustment of relevant constants) a decrease in the retention of dust in the lungs with a decrease in the cytotoxic effect of the same quartzite dust brought about by the administration of

(footnote continued)

literature including publications [12–18], or an permanently operating auxiliary phagocytosis mechanism of particle elimination as we have proved repeatedly (starting with [19]), or, which is most likely, both in different ratios, the active involvement of neutrophil leukocytes in this process is beyond any doubt.

¹ Note that irrespective of whether or not the neutrophilic shift in the BALF cell population is a sign of inflammation as it is typically assumed in the

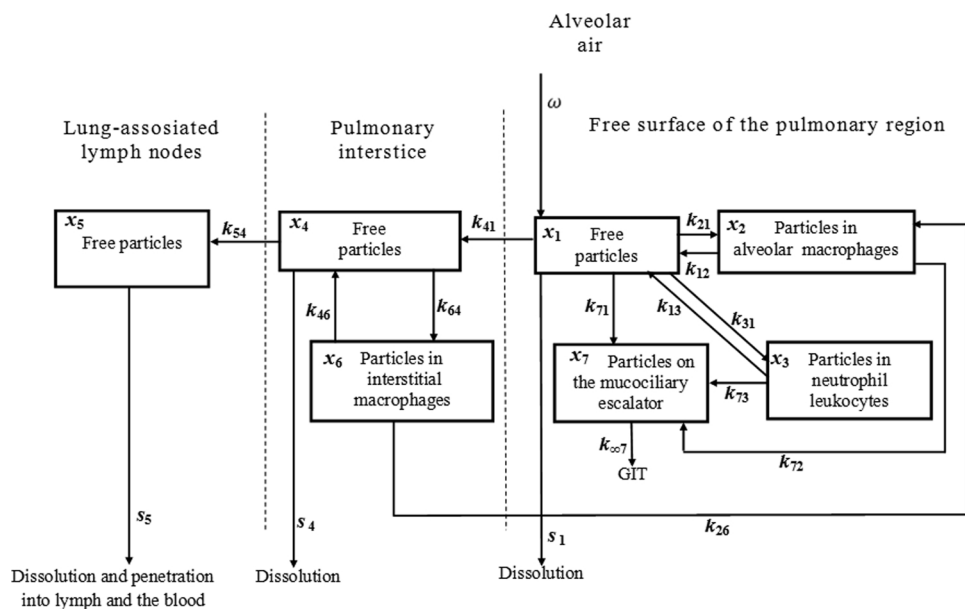


Fig. 3. The same model structure as in Fig. 1 – now allowing for the contribution of particle dissolution into the kinetics of pulmonary clearance; the variant employed in chronic inhalation experiments with nanoparticles of Fe₂O₃ [23] SiO₂ [24]. Notation of constants is explained in the Subsection 2.2.

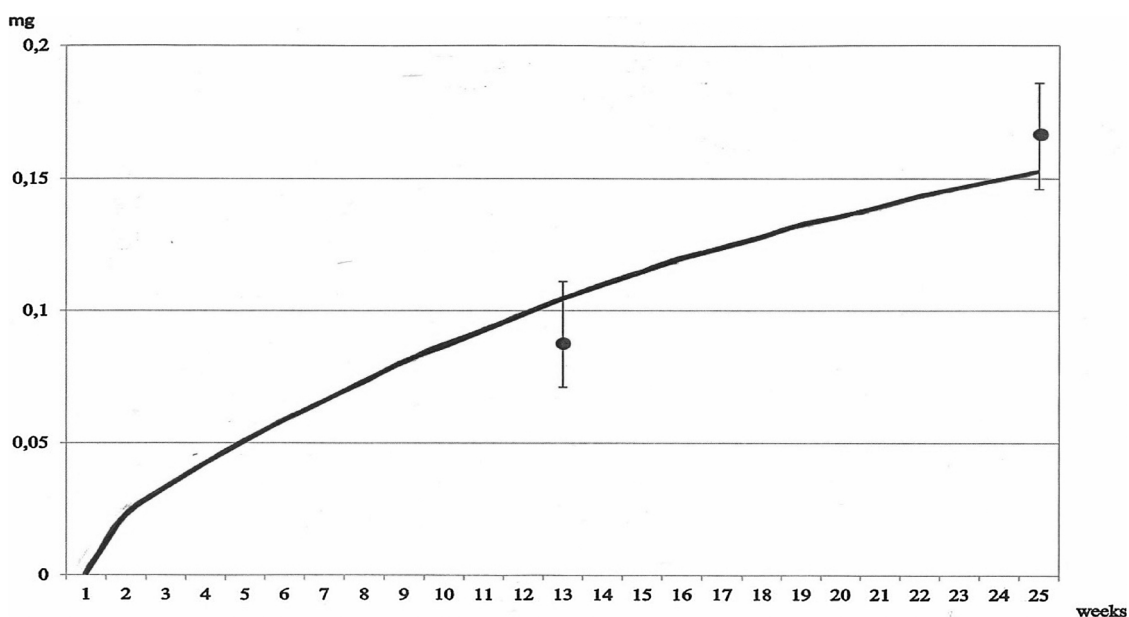


Fig. 4. The kinetics of SiO₂ accumulation in rat lungs as predicted by the model (Fig. 3) for a real exposure period and averaged experimental data with 95% CI. The abscissa plots the duration of the exposure period in weeks, and the ordinate, the SiO₂ content of lungs in mg [24].

2. Materials and methods

2.1. Experimental techniques

Airborne NiO-NPs were obtained by sparking from 99.99% pure nickel rods using the Palas DNP-3000 generator and fed into a nose-only computerized exposure device (CH Technologies, USA) for 60 rats placed into individual restrainers (Fig. 5). Particles collected on the polycarbonate filter and inspected under scanning electron microscope (SEM) had spherical form and either were singlet or formed small aggregates. The latter, if compact, were measured as one particle, and even with this assumption the particle size distribution (Fig. 6) proved rather clean-cut and restricted to the nanometric range with a mean (\pm s.d.) diameter of 23 ± 5 nm. The chemical identity of the

nanoparticles sampled on the filters was confirmed by the Raman spectroscopy as being NiO.

The experiment was carried out on outbred white female rats from our own breeding colony with the initial body weight of 150 to 220 g, with minimum 12 animals in a group investigated at a certain exposure term. Rats were housed in conventional conditions, breathed unfiltered air, and were fed standard balanced food. The experiments were planned and implemented in accordance with the “International guiding principles for biomedical research involving animals” developed by the Council for International Organizations of Medical Sciences (1985) and were approved by the Ethics Committee of the Ekaterinburg Medical Research Center Medical for Prophylaxis and Health Protection in Industrial Workers.

Rats were exposed for 4 h a day, 5 times per week, during 3, 6 or 10

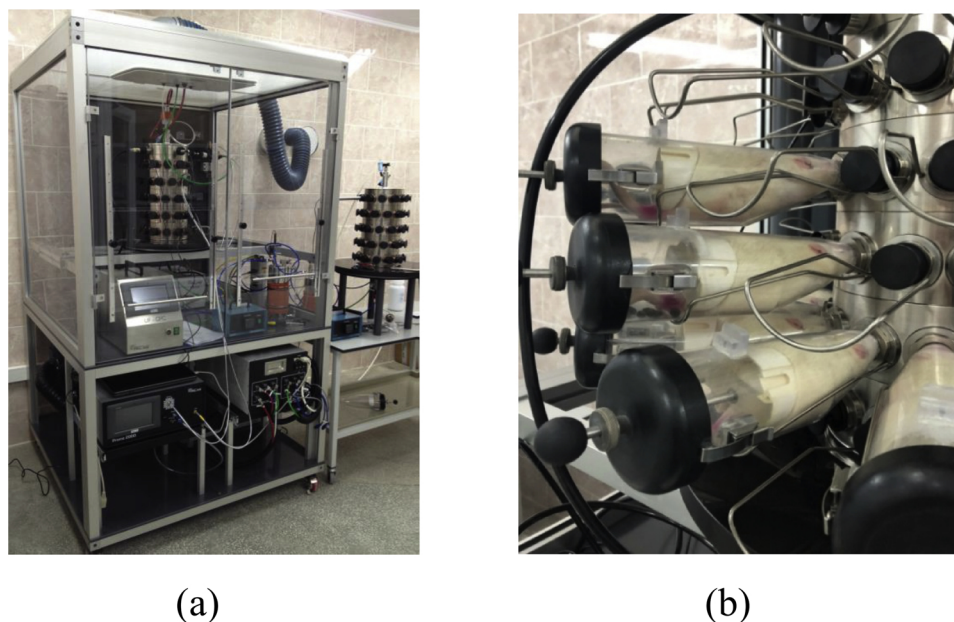


Fig. 5. (a) The nose-only inhalation device (photographed without door wings of the draught cupboard) and, in the background, a similar tower for sham exposure of control group; (b) restrainers with rats attached to the exposure tower.

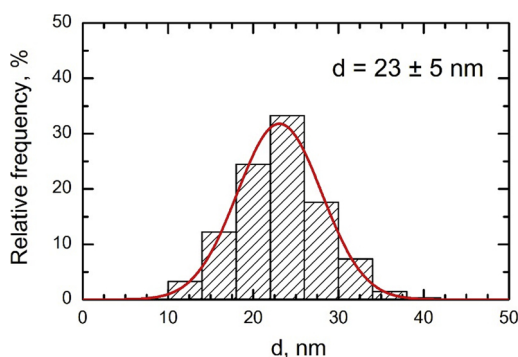


Fig. 6. Particle or particle-aggregate size distribution function obtained by statistical processing of 250 (N_0) measured SEM images of nanoparticles accumulated on a polycarbonate filter from the rat exposure breathing zone.

months at the average concentration of NiO-NP in the nose-breathing zone equal to $0.23 \pm 0.01 \text{ mg/m}^3$.² In 24 h after the final exposure of each of these periods, a bronchoalveolar lavage was performed for differential counting of cells in the lavage fluid (BALF). NiO contents were measured in the dry tissues of individual lungs and of pooled lung-associated lymph nodes by the Electron Paramagnetic Resonance Spectroscopy method (EPR) with a Bruker EMXplus EPR Spectrometer (USA). The same method was used for periodic measurements of the NiO-NP contents on pieces of the output filter of the inhalation setup after their incubation in deionized water, in BLAF supernatant or in fetal bovine serum (FBS) at 37°C for 4 days.

2.2. Identification of model parameters for particle redistribution, elimination and retention

The iterative mathematical procedure used for identifying

² Our intention was to carry out the experiment at a concentration of around 1 mg/m^3 , as in a previous similar study involving $\text{Fe}_2\text{O}_3\text{-NP}$ [22]. We found, however, that NiO-NP in this concentration caused marked signs of acute pulmonary toxicity precluding the possibility of performing any long-term exposure [25]

coefficients of differential equations which describes particulate flows between model's compartments or out of the modeled system was described in detail previously [7]. Let us but remind that in our model these coefficients are interpreted as particle transfer rate constants which are notated as k_{ji} for respective transfer from a compartment Xi to a compartment Xj or to outside "infinity" (∞) through the GIT. Later on, for the nanoparticles solubilization-due exits from a pulmonary compartment Xi into the circulation we introduced notation s_i .

Earlier we [23] so identified respective parameters of the multi-compartmental model for the retention of nanoparticles with reference to $\text{Fe}_2\text{O}_3\text{-NP}$ (Fig. 3) using the characteristics that had been proved reliable for modeling the retention of polydisperse dust particles of standard quartz DQ12 [9] and adjusting them in accordance with the outcomes of a comparative assessment of the BALF cellular response to intratracheal instillation of low doses of these two particulates and with the solubility of $\text{Fe}_2\text{O}_3\text{-NP}$ in model milieus. Proceeding now to modeling the chronic inhalation exposure of rats to NiO-NP, it was but natural to take as the new starting point just this variant of the model validated for $\text{Fe}_2\text{O}_3\text{-NP}$ and to adjust some of its parameters relying on similar comparative estimates of BALF cell counts and NP solubility. As always beginning from earliest works in this area, we adhered to a principle that it is inadmissible to fit a model prediction of retention to actual results by varying some other particle transfer rate constants for which no such experimental or, at least, reliable theoretical assumptions were available.

At the same time, we deem it possible to vary somewhat the mass deposition of particles in the lower airways (denoted as ω or ω_u in all charts of the model), since this quantity could be estimated only very roughly based on the following tentative physiological considerations:

- The rat's minute respiratory ventilation as assessed experimentally by different authors varies between 78 mL [26] and 210 mL [27]. The so-called Multi-Path Particle Dosimetry (MPPD) model for rats used in [28] assumes the breathing frequency to be equal 102 min^{-1} and tidal volume 2.1 mL which gives minute ventilation 214 mL which corresponds to upper and thus seems to be an extreme estimate. In the previous cases [23,24], we calculated particle deposition based on the minute ventilation value of 100 mL, which is within the above experimental range but of course somewhat arbitrary.

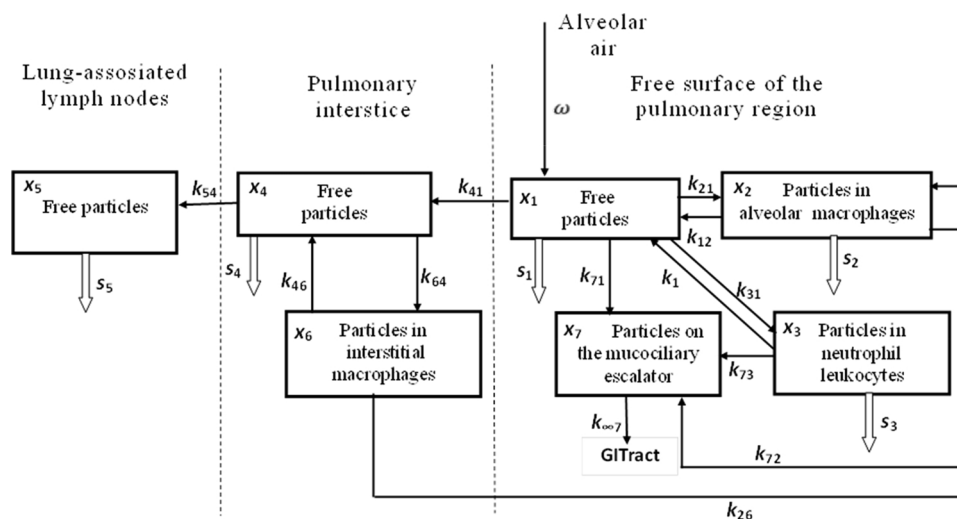


Fig. 7. The same model structure as in Fig. 4 – now with a specified contribution of nanoparticle dissolution to lung clearance kinetics. This variant was used in the chronic inhalation exposure experiments NiO-NP. Notation of constants is explained in the Subsection 2.2.

- (b) The pulmonary NP deposition fraction was assumed to be equal to 0.52, which is close to the lowest of the differently substantiated assessments given by [29] but much higher as compared with 0.124 assumed to be the alveolar region deposition fraction for 20 nm particles according to the same MPPD model [28].

Taking into consideration that not only singlet but even compact aggregates of the inhaled NPs were in the low nano-scale range, we believe that our estimate was not too high but again not an “exact” value and might be somewhat adjusted – especially if one takes into consideration that the mean NP diameters were different: 14 ± 4 nm for Fe_2O_3 -NP and 23 ± 5 nm for NiO-NP.

For model identification purposes we used as a statistical software the Mathcad 15.exe program.

3. Results and discussion

As was mentioned in the Introduction, we present here an experiment with NiO-NPs only with reference to a certain phase in the development of the mechanistic particle retention model which this paper is devoted to. A detailed description of the adverse effects resulting from inhalation exposure to these nanoparticles is the subject matter of special publications ([25] and more in press). Briefly, it was shown by us that the organism’s non-specific responses to the action of NiO-NPs includes: diverse manifestations of systemic toxicity with a particularly pronounced adverse influence on liver and kidney function, redox balance, damage to some areas of brain tissue (associated with proven transfer of nanoparticles from the nasal mucosa along the olfactory tract); some cytological signs for probable development of allergic syndrome; an exposure length dependent genotoxic effect even at the low exposure level at which systemic toxicity was rather modest; and a paradoxically low severity of pneumoconiosis type pathology explained by a very small chronic retention of nanoparticles in the lungs. Along with the above, NiO-NPs also induced phase-related stimulation of erythropoiesis, which is relatively specific for the nickel toxic effects.

As for the toxicokinetic model under present consideration, to be able to simulate the above-mentioned paradoxically low retention of nanoparticles in the lungs, it had to be modified as shown in Fig. 7. NiO-NP retention characteristics that justified such adaptation to be made and adaptation methods used are as follows:

1. NiO-NP solubilization in the organism (judging by the kinetics of dissolution “in vitro” – see Fig. 8) should be assumed to be even higher than that in the previous cases, although in this case as well it also had at least two phases: quick and slow.

Given such solubility, to use the previous model structure in which elimination due to this process was reduced to flows from only two lung compartments (Fig. 3) would require the flow rate constants to be assumed unrealistically high without any agreement with the quantitative estimates of solubility “in vitro”. Such wishful adjustment of the model could provide a desired result but would undermine our own trust in this model as a systems analysis tool. We therefore distributed the dissolution-associated elimination among a greater number of model compartments while allowing for the probability of intracellular solubilization of nanoparticles³.

2. In contrast to all our previous chronic inhalation experiments involving both dusts and nano-aerosols, the attainment of a plateau in the accumulation of the inhaled material in the NiO-NP experiment was not smooth in time but quite abrupt and virtually finished already by the end of earliest (3-months) assessment term. At a stable level of exposure, such leap-ahead retention kinetics could be possibly explained only by the assumption that in the still earlier phases the elimination of these NPs proceeded at substantially higher transfer rate constants followed by their reduction in either a stepwise or a smooth manner. The assumption of a stepwise reduction would require constructing two or more separate models of linear kinetics for different phases while the smooth one would require introducing into the model a certain mathematical function describing the decrease in its constants with time assuming a relatively quick attainment of a plateau by themselves. We have chosen the 2nd option.

At the same time, there are no sufficient grounds to assume such reduction in relation to the flow rate constants of the particle elimination that are due to their dissolution. On the other hand, it is quite admissible to assume an impairment to the protective and adaptive functional mechanisms caused by a continuing toxic impact on the organism. Indeed, the phagocytic response of the lungs to NiO-NP inhalation was rather pronounced during the three-month term and noticeably attenuated in the subsequent periods (Table 1). We may therefore assume that by the end of the three-month exposure period this response had already been a result of such reduction in the earlier period, at the beginning of which it was even more intense and effective.

Based on all these assumptions, we included into the mathematical model of NiO-NP retention in the lungs the functions $0.5 + .5^*e^{-.1*t}$

³ This process is generally recognized as one of the primary mechanisms of NP cytotoxicity but, as a rule, it is not taken into account as an elimination mechanism.

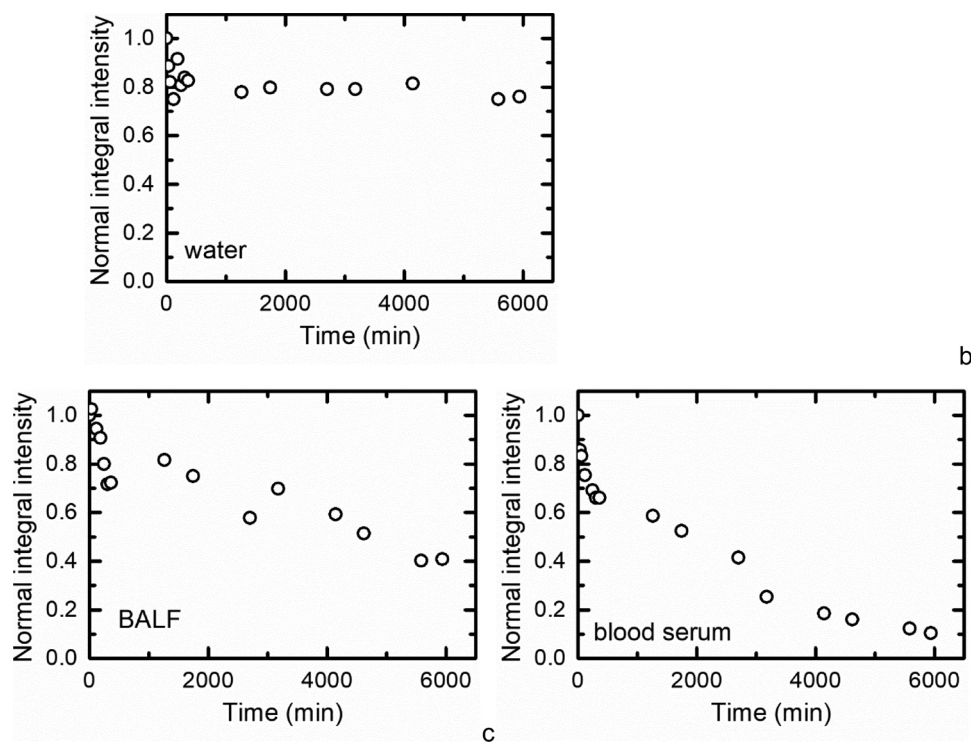


Fig. 8. The kinetics of reduction in the intensity of the EPR signal (in normalized arbitrary units) from NiO NPs collected on the output filter of the inhalation setup for incubation in (a) water or normal saline; (b) BALF supernatant; (c) sterile bovine blood serum.

Table 1

Cytological indices of the bronchoalveolar lavage fluid from rats exposed to chronic inhalation of NiO nanoparticles at a concentration of $0.23 \pm 001 \text{ mg/m}^3$, ($\bar{X} \pm S_x$).

Index	Duration of exposure period					
	3 months		6 months		10 months	
	Control	NiO NP	Control	NiO NP	Control	NiO NP
Total cell count $\times 10^6$	3.50 ± 0.66	21.40 $\pm 6.01^*$	2.94 ± 0.53	8.38 $\pm 0.81^*$	3.44 ± 0.46	9.63 $\pm 1.15^*$
Alveolar macrophages (AM) $\times 10^6$	3.06 ± 0.69	13.00 $\pm 3.22^*$	2.78 ± 0.53	6.67 $\pm 0.66^*$	3.14 ± 0.39	5.89 $\pm 0.70^*$
Neutrophil leukocytes (NL) $\times 10^6$	0.43 ± 0.18	8.40 $\pm 3.11^*$	0.27 ± 0.08	1.72 $\pm 0.26^*$	0.30 ± 0.08	3.74 $\pm 0.78^*$
NL/AM ratio	0.26 ± 0.18	0.61 ± 0.11	0.11 ± 0.04	0.26 $\pm 0.04^*$	0.09 ± 0.02	0.65 $\pm 0.12^*$

Note: * – a statistically significant difference from the “control” group (for $p \leq 0.05$ by Student’s *t*-test).

and $0.15 + 1.35 \cdot e^{-1.1t}$ instead of the transfer rate constant values identified for the model of retention of Fe_2O_3 -NPs for transfer from the free particles compartment to the compartments corresponding, respectively, to the pools of particles phagocytized by alveolar macrophages ($k_{21} = 0.5$) and neutrophil leukocytes ($k_{31} = 0.15$).

As a result of all of these transformations, the model prediction for the retention of NiO- NPs in the lungs fits well enough the experimental results in relation to both the relatively low height of the plateau and the unusually high speed of its attainment (Fig. 9). Note that the equilibrium level of retention predicted by the model by the end of the ten-months experiment lies within the 95% confidence interval for the mean value of actual accumulation of nanoparticles in the lungs.

It should be mentioned in this context that not only phagocytic clearance mechanisms may be subject to cytotoxic damage by NPs but also their translocation from alveoli to lung interstices which is

described in our model as a passive diffusion process but is in fact controlled, even if partially, controlled by cells forming the barrier between these compartments [30]. In fact, it has been already stressed by us that “we should try and incorporate into this model the possible toxicokinetic repercussions of the NP-induced damage to alveolocytes” [23] but this is still to be done.

As to the practical significance of our work, we’d like to stress again that the main goal of developing so-called “models of systems” (as distinct from “models of data”) is not to directly simulate real-life situations but to strengthen theoretical foundations on which such simulations and predictions are based. Thus, for example, although toxicologists usually predict especially high human health risks associated with nanoparticles exposures, our model proves that it may be not absolutely right when dealing with inhalation exposure.

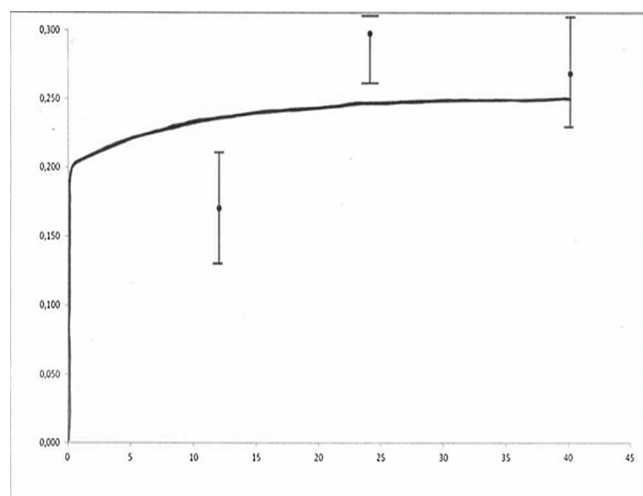


Fig. 9. The kinetics of NiO-NP accumulation in rat lungs as predicted by the model (Fig. 7) for a real exposure period (line) and the mean experimental data (dots with 95% CI). The abscissa plots the duration of the exposure period in weeks, and the ordinate plots the NiO-NP content of the lungs in mg.

4. Conclusions

Thus, the multicompartmental model for the retention of particles in lungs under chronic inhalation which was first developed for various conditions of exposure to practically insoluble polydisperse mineral dusts has by now been successfully used as a basic model for analyzing patterns in the retention of oxide nanoparticles of three various chemical elements (iron, silicon and nickel).

The most significant adjustment of this model to the conditions of exposure to these nanoparticles was required by the need to mathematically describe not only the physiological mechanisms of their elimination from the lungs and intrapulmonary translocation but also the kinetics of their dissolution “in vivo”, while the relative contribution of the latter may be different for nanoparticles of different chemical nature.

Damage to the above physiological mechanisms by highly toxic nanoparticles or impairment of these mechanisms as a result of changes in the general protective reactivity of the organism under chronic intoxication may explain a non-linear character of the kinetics of retention of such particles.

In our opinion, a satisfactory effectiveness of the model under consideration which proved to be easily adaptable to variable conditions of chronic inhalation exposure to diverse particulates in both micro- and nano-size ranges, may be considered as a testimony to the adequacy of the theoretical mechanistic premises based on which this model has been developed.

Transparency document

The [Transparency document](#) associated with this article can be found in the online version.

References

- [1] A.M. Tsatsakis, D. Kourtas, M.N. Tzatzarakis, P. Stivaktakis, K. Tsarouhas, K.S. Golokhvast, V.N. Rakitskii, V.A. Tutelyan, A.F. Hernandez, R. Rezaee, G. Chung, C. Fenga, A.B. Engin, M. Neagu, A.L. Arsene, A.O. Docea, E. Gofita, D. Calina, I. Taitzoglou, J. Liesivuori, A.W. Hayes, S. Gutnikov, C. Tsitsimpikou, Simulating real-life exposures to uncover possible risks to human health: a proposed consensus for a novel methodological approach, *Hum. Exp. Toxicol.* 36 (6) (2017) 554–564.
- [2] A.M. Tsatsakis, L. Vassilopoulou, L. Kovatsi, C. Tsitsimpikou, M. Karamanou, G. Leon, J. Liesivuori, A.W. Hayes, D.A. Spandidos, The dose response principle from philosophy to modern toxicology: the impact of ancient philosophy and medicine in modern toxicology science, *Toxicol. Rep.* 6 (5) (2018) 1107–1113.
- [3] J.D. Brain, P.A. Valberg, Models of lung retention based on the ICRP task group report, *Arch. Environ. Health* 28 (1) (1974) 1–11.
- [4] E. Fröhlich, S. Salar-Behzadi, Toxicological assessment of inhaled nanoparticles: role of *in vivo*, *ex vivo*, *in vitro*, and *in silico* studies, *Int. J. Mol. Sci.* 15 (2014) 4795–4822.
- [5] I.A. Minigalieva, B.A. Katsnelson, V.G. Panov, A.N. Varaksin, V.B. Gurvich, L.I. Privalova, M.P. Sutunkova, S.V. Klinova, Experimental study and mathematical modeling of toxic metals combined action as a scientific foundation for occupational and environmental health risks assessment (a synthesis of results obtained by the Ekaterinburg research team, Russia), *Toxicol. Rep.* 4 (2017) 194–201.
- [6] R.N. Kostoff, M. Goumenou, A. Tsatsakis, The role of toxic stimuli combinations in determining safe exposure limits, *Toxicol. Rep.* 7 (5) (2018) 1169–1172.
- [7] B.A. Katsnelson, L.I. Privalova, L.K. Konyshva, K.I. Morosova, Development of a multicompartmental model of the kinetics of quartz dust in the pulmonary region of the lung during chronic inhalation exposure of rats, *Br. J. Ind. Med.* 49 (3) (1992) 172–181.
- [8] B.A. Katsnelson, L.K. Konyshva, N.Ye. Sharapova, L.I. Privalova, Prediction of the comparative intensity of pneumoconiotic changes caused by chronic inhalation exposure to dusts of different cytotoxicity by means of a mathematical model, *Occup. Environ. Med.* 5 (3) (1994) 173–180.
- [9] B.A. Katsnelson, L.K. Konyshva, L.I. Privalova, N. Ye, Sharapova quartz dust retention in rat lungs under chronic exposure simulated by a multicompartmental model: further evidence of the key role of the cytotoxicity of quartz particles, *Inhal. Toxicol.* 9 (7) (1997) 703–715.
- [10] W. Stöber, POCK model simulations of pulmonary quartz dust retention data in extended inhalation exposures of rats, *Inhal. Toxicol.* 11 (4) (1999) 269–292.
- [11] L. Tran, D. Buchanan, B.G. Miller, K. Donaldson, Mathematical modeling to predict the responses to poorly soluble particles in rat lungs, *Inhal. Toxicol.* 12 (3) (2000) 403–409.
- [12] L. Renwick, D. Brown, K. Clouter, K. Donaldson, Increased inflammation and altered macrophage chemotactic responses caused by two ultrafine particle types, *Occup. Environ. Med.* 61 (2004) 442–447.
- [13] T. Stoeger, C. Reinhard, Sh. Takenaka, A. Schroepel, E. Karg, B. Ritter, Instillation of six different ultrafine carbon particles indicates a surface area threshold dose for acute lung inflammation in mice, *Environ. Health Perspect.* 114 (3) (2006) 328–333.
- [14] T.M. Sager, D.W. Porter, V.A. Robinson, W.G. Lindsley, V.A. Schwegler-Berry, V. Castranova, Improved method to disperse nanoparticles *in vitro* and *in vivo* investigation of toxicity, *Nanotoxicology* 1 (2007) 118–129.
- [15] V.H. Grassian, P.T. O’Shaughnessy, A. Adamcakova-Dodd, J.M. Pettibone, P.S. Thorne, Inhalation exposure study of titanium dioxide nanoparticles with a primary particle size of 2 to 5 nm, *Environ. Health Perspect.* 115 (2007) 397–402.
- [16] M. Neuberger, *Umweltepide miologie und toxi kologie von nanopartikeln*, in: A. Gazsó, S. Greßler, F. Schiemer (Eds.), *Nano-Chancen und Risiken aktueller Technologien*, Springer, Wien - New York, 2007, pp. 181–197.
- [17] D.B. Warheit, K.L. Reed, C.M. Sayes, A role for surface reactivity in TiO₂ and quartz-related nanoparticle pulmonary toxicity, *Nanotoxicology* 3 (2009) 181–187.
- [18] J. Liu, X. Feng, L. Wei, L. Chen, B. Song, L. Shao, The toxicology of ion-shedding zinc oxide nanoparticles, *Crit. Rev. Toxicol.* 46 (4) (2016) 348–384.
- [19] L.I. Privalova, B.A. Katsnelson, A.B. Osipenko, B.N. Yushkov, L.G. Babushkina, Response of a phagocyte cell system to products of macrophage breakdown as a probable mechanism of alveolar phagocytosis adaptation to deposition of particles of different cytotoxicity, *Environ. Health Perspect.* 35 (1980) 205–218.
- [20] B. Bellmann, H. Muhle, O. Creutzenberg, C. Dasenbrock, R. Klipper, J.C. MacKenzie, P. Morrow, R. Mermelstein, Lung clearance and retention of toner, utilizing tracer technique during chronic inhalation exposure in rats, *Fundam. Appl. Toxicol.* 17 (1991) 300–313.
- [21] B.A. Katsnelson, L.I. Privalova, S.V. Kuzmin, T.D. Degtyareva, M.P. Sutunkova, O.S. Yermenko, Some peculiarities of pulmonary clearance mechanisms in rats after intratracheal instillation of magnetite (Fe₃O₄) suspensions with different particle sizes in the nanometer and micrometer ranges: Are we defenseless against nanoparticles? *Int. J. Occup. Environ. Health* 16 (2010) 508–552.
- [22] M.P. Sutunkova, L.I. Privalova, I.A. Minigaliyeva, V.B. Gurvich, V.G. Panov, B.A. Katsnelson, The most important inferences from the Ekaterinburg nanotoxicology team’s animal experiments assessing adverse health effects of metallic and metal oxide nanoparticles, *Toxicol. Rep.* 5 (2018) 363–376.
- [23] M.P. Sutunkova, B.A. Katsnelson, L.I. Privalova, V.B. Gurvich, L.K. Konyshva, V. Ya, E.V. Shishkina Shur, I.A. Minigalieva, S.N. Solovjeva, S.V. Grebenkina, I.V. Zubarev, On the contribution of the phagocytosis and the solubilization to the iron oxide nanoparticles retention in and elimination from lungs under long-term inhalation exposure, *Toxicology* 363 (2016) 19–28.
- [24] S.N. Solovjeva, M.P. Sutunkova, B.A. Katsnelson, V.B. Gurvich, L.I. Privalova, I.A. Minigalieva, T.V. Slyshkina, I.E. Valamina, O.H. Makeyev, V.Ya. Shur, I.V. Zubarev, D.K. Kuznetsov, E.V. Shishkina, Interplay of the pulmonary phagocytosis response to, and the *in vivo* solubilization of amorphous silica nanoparticles deposited in lungs of rats under long-term inhalation exposures as determinants of their modest fibrogenicity and low systemic toxicity (experimental and mathematical modeling), Abstract: International Conference on Occupational Safety and Health, 4th Edition, London, U.K. (2018).
- [25] B.A. Katsnelson, M.P. Sutunkova, L.I. Privalova, S.N. Solovjeva, V.B. Gurvich, T.V. Bushueva, R.R. Sakhaudina, I.E. Valamina, O.H. Makeyev, I.V. Zubarev, I.A. Minigalieva, S.V. Klinova, V.Ya. Shur, Yu.V. Gribova, A.E. Tsaregorodtseva, A.V. Korotkov, E.A. Shuman, E.V. Shishkina, Toxic effects assessment of nickel oxide nanoparticles by inhalation, *Popul. Health Habitat* 309 (12) (2018) 24–29 (in Russian).

- [26] G. Ramahandran, *Assessing Nanoparticle Risk to Human Health*, Elsevier, Amsterdam, 2016.
- [27] J.L. Maulderly, R.G. McCunney, *Particle Overload in the Rat Lung and Lung Cancer. Implications for Human Risk Assessment*, Taylor & Francis, Philadelphia, USA, 1997.
- [28] D.S. Anderson, E.S. Patchin, R.M. Silva, D.L. Uyeminami, A. Sharmah, T. Guo, G.K. Das, J.M. Brown, J. Shannahan, T. Gordon, Chen Lung Chi, K.E. Pinkerton, L.S. Van Winkle, Influence of particle size on persistence and clearance of aerosolized silver nanoparticles in the rat lung, *Toxicol. Sci.* 144 (2) (2015) 366–381.
- [29] A.V. Kolanjiyil, *Deposited Nanomaterial Mass Transfer From Lung Airways to Systemic Regions. A Thesis for MSc Degree*, Raleigh, NC (2013).
- [30] S. Dekali, C. Gamez, T. Kortulewski, K. Blazy, P. Rat, G. Lacroix, Assessment of an in vitro model of pulmonary barrier to study the translocation of nanoparticles, *Toxicol. Rep.* 1 (2014) 157–171.