Critical Experimental Conditions for Testing Combustion Generated Nanoparticles

J. O. BRUCH; IBE Inst. für biologische Emissionsbewertung MARL;

Joachim.Bruch@ibe-marl.de

The need to evaluate the amount of toxicity of inhaled particles arises from clinical and epidemiological experience that occupational exposure to mineral dusts causes extremely vast differences in the extent of risk. For crystalline silica exposure at different mines and worksites the risk varies by about 1:10, if particles with low biological activity are included the risks differences sum up to 1:30. Experimental tests have been established in which key steps of the disease development were taken to assess the amount of toxicity and the no effect exposure level (NEL). For instance expression of oxidative stress (OS) turned out to be a leading mechanism of the toxicity of poorly soluble particles (PSP), such as respirable mineral particles or aggregates of metal oxide or carbon nanoparticles (NP). In the sequence of damaging, scavenging and repair the amount of oxidized DNA (8-oxoGua; etheno-adducts) sums up all previous reactions and can be taken as an interim endpoint for persisting OS. A critical methodological element is the multi-dose exposure scheme including the NEL in the non-overload domain. Originally proposed by Morrow and confirmed experimentally, the volumetric overload starts at 6% of the alveolar macrophage (AM) volume or 120 pg/rat AM, or in intratracheal instillation (ITI) studies at not more than 4.8 mg / rat lung (b.w. 180g).

Overload studies have been the source of many unspecific results in particle research, mainly inflammation, mutagenicity and cancerogenicity (Valberg et al., 2009). High doses elicit new effect mechanisms, which are irrelevant for human endangerment (Slikker et al., 2004).

In nanotoxicology the evaluate scheme for analyzing health related bio-effects of nanoparticles (NP) are under discussion. Controversial issues are the choice of appropriate test systems for checking for important factors of NP effect mechanisms. The very small size of the particles and the huge surface of the NPs were regarded as decisive characteristics accounting for the particular toxicity of NP. The NPs are thought to escape the AM phagocytic activity and to migrate through the epithelial barrier into the interstitial tissue. Further studies also speculate on the systemic penetration of NP.

The surface of NPs matters since

- the particular constitution of the presented structure attracts specifically relevant biomolecules from the microenvironment and
- the amount and quality of adsorbed chemicals in combustion generated NP (CGNP) attribute to the bioactivity of the NP.

Thus the appropriate design of the test models addressing size and surface of the NP as well as bioavailability of adsorbed chemicals of the CGNP appears meaningful.

Inhalation experiments are considered as gold standard for assessing relevant particle lung effects such as inflammation, fibrosis, OS and allergic response etc.. Alternatively ITI administration in the non-overload domain may help to differentiate acute from chronic effects. In the NanocareProject (NCP) a wide range of test methods has been analyzed in assaying 12 different engineered NPs (ENPs) including animal exposure by inhalation (1), ITI (2), in vitro tests on ex vivo AM, the vector model (VM) (3) and in

using cell lines (4). Here the methods (1) to (3) confirmed the necessity of testing in the non-overload domain also in the field nanotoxicology; the obtained data (from 1, 2, 3) were consistent and mutually confirmative.

Animal experiments are expensive; they are restricted by national agencies (e.g. the EU "3R" initiative: refinement, restriction and replacement of animal experiments). Thus the rapid rising numbers of ENPs, as well as the environmental ultra fine particles (UFP) require efficient high through put (HPT) in vitro test procedures. UFP are thought to increase cardio-vascular diseases (CVDs), respiratory diseases including lung cancer; the differences in toxicity of the UFPs from different sources in the environment (rural vs. metropolitan areas) exceed the range of 1:100 (according to our in vitro data, multi-dose and NELs taken as a measure).

Of course those in vitro assays reduce to some extent the complex interactions of in vivo processes, however the capability to test for important known pathogenic mechanisms is indispensable. Discriminatory test power is desirable with regard to the paradigms of NP-toxicity such as size, surface and specific bioactivity of the surface of the ENP.

A critical issue is the physico-chemical characterization of the test material being present in the assays aimed to check for the relevant endpoints. Exposure conditions in vitro are predetermined by the requirements of the cell test system in use. For instance the - apostrophized *human pneumocyte type II* - cell line A549 (like many other cell lines) needs 5-10% FCS in the culture medium, where as ex vivo alveolar macrophages can be cultivated in balanced salt solutions (e.g. MEM) possibly supplemented with 0.025% DPPC (a component of the phospholipids of lung surfactant). The dispersity of the agglomerations of the NM samples depends on the zeta-potential at the NM surface. The NCP thoroughly analyzed the size distributions of the 12 samples of ENP both airborne and in the different conditions being present in in vitro tests on cells. In physiological salt solutions (eg. Dulbecco's Modified Eagle's Medium (DMEM)) „all of the tested NPs were strongly agglomerated and no significant fraction of ultra fine particles could be determined ... . With increasing concentration of FCS, the mean particle size distribution (d50) decreased, and a significant effect could be seen even in the presence of 5% FCS" (Schulze et al., 2008). Thus agglomerations and grain sizes of the ENP were widely different in both in vitro systems for instance testing with A549 vs. ex vivo AM. The phospholipids of the lung surfactant do not de-agglomerate ultrafine TiO_2 (p25 Evonik) (Maier et al., 2006). Inhalation studies and TEM analysis on TiO_2 (van Ravenzwaay et al., 2009) or AlOOH (Pauluhn, 2009) of the NCP project showed that the visible amount of deposited ENP was agglomerated and incorporated in AM. Interestingly overload conditions only lead to a measurable drain of the ENM into the LN (Pauluhn, 2009); the TEM pictures on the lymph nodes of the overloaded TiO_2 study presented only agglomerated TiO_2 and no singlet particles (van Ravenzwaay et al., 2009). There are other studies on TiO_2, which possibly yield different data. It should be noted, however, that nanoparticles produced by the spark generator should not be confused with ENP nanoparticle.

The next critical point is the interaction of ENP surface with biological substrates and the resulting toxicological effects. Already in the 80s working groups in the NIOSH, WV, demonstrated the eminent influence of surface adsorbed biomolecules on the toxicity of the particles. According to the experimental experiences of Wallace and co-workers (Wallace et al., 2006):
„Quartz and kaolin dust prompt in vitro induction of LDH release from macrophage is suppressed in 10% fetal bovine serum (FBS) medium ...This indicates that short-term in vitro assay results can be affected by assay system nutrients that are not necessarily representative of in vivo pulmonary hypophase exposures." Similar inhibitory effects could be observed also by a high exposure of the particles to surfactant lipids. However

the in vitro studies in the VM using a very low 0.025 DPPC supplement are not affected according to our systematic analysis.

A critical issue for the experimental conditions in vitro and in vivo is the different specific power of attraction of ENP surface for biomolecules. Studies in the NCP indicate different forces in binding proteins, in particular SP-A, by incubating the ENP of the NCP with porcine broncho-alveolar lavage fluid. The IBE studies showed in analyzing lung function on an ex vivo lung model that instilled particles with different grain sizes adsorb functional surfactant components, an effect which could be compensated by an additional dose of DPPC (see folia) (Wiemann et al., 2010). Importantly, the attractive force at ENP for surfactant phospholipids is noticeably dissimilar for different samples with identical BET surfaces (see folia). In the NCP the results of the cell line tests are widely divergent from the results on ex vivo AM in the VM. Unfortunately, the different sizes (agglomeration) of the tested ENP are determined by the obligatory culture media as well as the different adsorption of blocking biomolecules.

Considerable experimental efforts are underway to avoid in some part the dilemmas of submersed in vitro test systems, such as intricate macrophage-epithelia cell-systems with air-liquid exposure, eventually being supplemented by adding dendritic cells to the system (Rothen-Rutishauser et al., 2005; Lenz et al., 2009; Lehmann et al., 2011). In this context it is important to note that all cell lines in vitro, spread out on culture bottom, easily incorporate particles; question of comparable exposures to in vivo situation are critical (first publication in my research group (Beck et al., 1964)).

Taken together uncertainties exist about the understanding of the toxicity results of many past toxicological studies on ENM. Some studies have limited interpretability because material characteristics (surface charge, agglomeration) have not always been fully measured or reported under the test conditions used. For instance animal studies evidencing higher inflammatory effects of ultrafine TiO_2 (-particles) compared to pigmentary TiO_2 possibly reflect the effects of different surfaces of the NM rather than the effects of the small size of particles as such (compare the data of v. R. (van Ravenzwaay et al., 2009).

Validation of cell line data to animal results is desirable; attempts so far have not been successful (Sayes et al., 2006; Warheit et al., 2007). Yet the assays on ex vivo macrophages as used in the VM were corroborated by the in vivo ITI assays as well as by the inhalation experiments (Bruch et al., 2009).

The selectivity and specificity of the binding of surfactant components poses the speculative question as to how ENP bound functional molecules were regulated in the intra-alveolar milieu. Adsorption of SP-A might be meaningful for immunological processes (Schulze et al., 2011). Studies on the interaction of NP with model lung surfactant monolayer indicate specific action on critical domains; lipid-layers containing SP-C were considerably affected by NP (Harishchandra et al., 2010).

Critical experimental conditions in testing CGNP

Combustion generated nanoparticles pose an even higher challenge to the experimental setting. Relevant exposure to CGNP is ubiquitous in urban environment (pm2.5, ultrafine particles, (UFP)) with high pollution from traffic (diesel emissions particles, DEPs) as well as from coal burning power plants and other sources of anthropogenic activities.

The inorganic fraction of the particulate phase of CGNP primarily consists of small elemental carbon particles ranging from 10 to 40 nm diameter forming aggregates of about 60 – 100 nm. Because of their high surface area, CGNP are capable of adsorbing

relatively large amounts of organic material (organic compounds identified in diesel exhaust emissions contain hydrocarbons, hydrocarbon derivatives, PAHs, PAH derivatives, multifunctional derivatives of PAHs, a variety of which are mutagens and carcinogens such as PAH and nitro-PAH).

A plethora of experimental studies showing adverse effects of the chemist compounds isolated from the DEPs or environmental UFPs, many of them point to the mutagenicity of the lipophilic substances, others give evidence for a specific toxicity of the transitions metals; on molecular level OS is thought as important mechanism for both kinds of chemist compounds and for the effects of the UFP.

However the relative importance of the findings for acute and chronic effects in humans is debated since adverse exposure levels for diseases are observed at about 30 $\mu g/m^3$, and acute exposures (2 hrs) of healthy volunteers induce inflammatory reaction in the respiratory tract even at about 110 $\mu g/m^3$ DEP (together with the gaseous diesel emission) (Stenfors et al., 2004). In particular the patho-mechanisms for the manifestation and aggravation of cardiovascular diseases are disputed.

In assaying the kind and amount of toxicity of the various types of UFP (CNGP, DEP, ROFA, etc.) the contribution of the different sources to the risks would be analyzed; eventually "air toxics hot spots" could be identified. The experimental conditions in in vitro test for the examination of the CGNP such as DEP should enable the disposal of the relevant substances adsorbed at the surface. Experimental data disclose that lung surfactant adsorbs also on UFP/DEP (Kendall et al., 2004a; Kendall et al., 2004b). Furthermore the adsorption at the surface persists after incorporation in cells (Wallace et al., 2006). The NIOSH group could evidence that the in in vitro test on mutagenicity of DEP the intracellular disposal of the surface bound PAH is provided by lung surfactant lipids (Wallace et al., 2006). In inhalation studies with DEP Gerde (Gerde et al., 2001) found that a considerable amount of PAH is rapidly bioavailable within 30 min due to the mechanism of lateral diffusion (Bruch, pers. opinion 2011). Surfactant is the likely prime agent for particle-associated PAH extraction in the alveolar region. Recently the interaction of benzo[a]pyrene and DEP with the lung surfactant was analyzed on the structural-molecular level (Sosnowski et al., 2011). Taken together circumstantial evidence underline the eminent role of lung surfactant for the disposal of PAHs to biological systems. Regarding the relative toxicological importance of the organic chemicals, represented by PAHs, the study of Delfino et al. shows that the organic chemicals, represented by PAHs is associated with increased systemic inflammation (incl. CVDs) and explain associations with quasi-ultrafine particle mass (Delfino et al., 2005).

Transition metals are also suspected to contribute to the adverse effect of CGNP or environmental UFP (Adamson et al., 1999; Adamson et al., 2000; Prieditis and Adamson, 2002; Adamson et al., 2004). Actual toxicological results point to a specific cardio-toxic effect of particulate matter-associated zinc (Kodavanti et al., 2008). High pneumo-toxic capacity of this metal, contained ubiquitous in pm2.5, has been determined also by others (eg. (Adamson et al., 2000)).

An essential methodological issue is the rapid disposal of both types of substances (PAH + LSF, metals) to biological test systems. Zinc-oxid adsorbed on UFP could be easily lost under unfavorable conditions in sampling, storing and dosing. The other point is the fast development of inflammatory response (2-12 hrs) and the rapid decline of the "first-line-adverse effects" such as membrane leakage or PMN in the BAL thus indicating a "rapid recovery". Whereas appropriate parameters like tests for interstitial fibrosis or oxidative DNA-adduct potentially indicate persisting damage, according to our own experiences (report is ready; to be published).

Literature

Adamson IY, Prieditis H, Hedgecock C, Vincent R. 2000. Zinc is the toxic factor in the lung response to an atmospheric particulate sample. Toxicol Appl Pharmacol 166:111-119.

Adamson IY, Prieditis H, Vincent R. 1999. Pulmonary toxicity of an atmospheric particulate sample is due to the soluble fraction. Toxicol Appl Pharmacol 157:43-50.

Adamson IY, Prieditis H, Vincent R. 2004. Soluble and insoluble air particle fractions induce differential production of tumor necrosis factor alpha in rat lung. Exp Lung Res 30:355-368.

Beck EG, Santer A, Brockhaus A. 1964. [Biochemical Study of the Effect of the Cytostatic Quartz Action through 3 Low-Molecular Pyridine-N-Oxide Compounds on an Established Cell Strain (L929)]. Z Naturforsch B 19:1048-1054.

Bruch J, Landsiedel R, Ma-Hock L, Pauluhn J, Ragot J, Wiemann M. 2009. In vivo Test Systems. In: T.A.J. Kuhlbusch I, Duisburg, Germany H.F. Krug, Empa, St. Gallen, Switzerland K. Nau, Forschungszentrum Karlsruhe, Germany, editor. NanoCare: Health related Aspects of Nanomaterials. Frankfurt: DECHEMA e.V., D-60486 Frankfurt am Main Germany, 2009. p 48-62.

Delfino RJ, Sioutas C, Malik S. 2005. Potential role of ultrafine particles in associations between airborne particle mass and cardiovascular health. Environ Health Perspect 113:934-946.

Gerde P, Muggenburg BA, Lundborg M, Dahl AR. 2001. The rapid alveolar absorption of diesel soot-adsorbed benzo[a]pyrene: bioavailability, metabolism and dosimetry of an inhaled particle-borne carcinogen. Carcinogenesis 22:741-749.

Harishchandra RK, Saleem M, Galla HJ. 2010. Nanoparticle interaction with model lung surfactant monolayers. J R Soc Interface 7 Suppl 1:S15-26.

Kendall M, Brown L, Trought K. 2004a. Molecular adsorption at particle surfaces: a PM toxicity mediation mechanism. Inhalation Tox 16(S1)

:99-105.

Kendall M, Guntern J, Lockyer NP, Jones FH, Hutton BM, Lippmann M, Tetley TD. 2004b. Urban PM2.5 surface chemistry and interactions with bronchoalveolar lavage fluid. Inhal Toxicol 16 Suppl 1:115-129.

Kodavanti UP, Schladweiler MC, Gilmour PS, Wallenborn JG, Mandavilli BS, Ledbetter AD, Christiani DC, Runge MS, Karoly ED, Costa DL, Peddada S, Jaskot R, Richards JH, Thomas R, Madamanchi NR, Nyska A. 2008. The role of particulate matter-associated zinc in cardiac injury in rats. Environ Health Perspect 116:13-20.

Lehmann AD, Daum N, Bur M, Lehr CM, Gehr P, Rothen-Rutishauser BM. 2011. An in vitro triple cell co-culture model with primary cells mimicking the human alveolar epithelial barrier. Eur J Pharm Biopharm 77:398-406.

Lenz AG, Karg E, Lentner B, Dittrich V, Brandenberger C, Rothen-Rutishauser B, Schulz H, Ferron GA, Schmid O. 2009. A dose-controlled system for air-liquid interface cell exposure and application to zinc oxide nanoparticles. Part Fibre Toxicol 6:32.

Maier M, Hannebauer B, Holldorff H, Albers P. 2006. Does lung surfactant promote disaggregation of nanostructured titanium dioxide? J Occup Environ Med 48:1314-1320.

Pauluhn J. 2009. Pulmonary toxicity and fate of agglomerated 10 and 40 nm aluminum oxyhydroxides following 4-week inhalation exposure of rats: toxic effects are determined by agglomerated, not primary particle size. Toxicological sciences : an official journal of the Society of Toxicology 109:152-167.

Prieditis H, Adamson IY. 2002. Comparative pulmonary toxicity of various soluble metals found in urban particulate dusts. Exp Lung Res 28:563-576.

Rothen-Rutishauser BM, Kiama SG, Gehr P. 2005. A three-dimensional cellular model of the human respiratory tract to study the interaction with particles. Am J Respir Cell Mol Biol 32:281-289.

Sayes CM, Wahi R, Kurian PA, Liu Y, West JL, Ausman KD, Warheit DB, Colvin VL. 2006. Correlating nanoscale titania structure with toxicity: a cytotoxicity and inflammatory response study with human dermal fibroblasts and human lung epithelial cells. Toxicol Sci 92:174-185.

Schulze C, A. Kroll, C.M. Lehr, U.F. Schaefer, K. Becker, J. Schnekenburger, C. Schulze Isfort, R. Landsiedel, Wohlleben W. 2008. Not ready to use overcoming pitfalls when dispersing nanoparticles in physiological media. Nanotoxicology 2:51-61.

Schulze C, Schaefer UF, Ruge CA, Wohlleben W, Lehr CM. 2011. Interaction of metal oxide nanoparticles with lung surfactant protein A. European journal of pharmaceutics and biopharmaceutics 77:376-383.

Slikker W, Jr., Andersen ME, Bogdanffy MS, Bus JS, Cohen SD, Conolly RB, David RM, Doerrer NG, Dorman DC, Gaylor DW, Hattis D, Rogers JM, Woodrow Setzer R, Swenberg JA, Wallace K. 2004. Dose-dependent transitions in mechanisms of toxicity. Toxicol Appl Pharmacol 201:203-225.

Sosnowski TR, Kolinski M, Gradon L. 2011. Interactions of benzo[a]pyrene and diesel exhaust particulate matter with the lung surfactant system. The Annals of occupational hygiene 55:329-338.

Stenfors N, Nordenhall C, Salvi SS, Mudway I, Soderberg M, Blomberg A, Helleday R, Levin JO, Holgate ST, Kelly FJ, Frew AJ, Sandstrom T. 2004. Different airway inflammatory responses in asthmatic and healthy humans exposed to diesel. Eur Respir J 23:82-86.

Valberg PA, Bruch J, McCunney RJ. 2009. Are rat results from intratracheal instillation of 19 granular dusts a reliable basis for predicting cancer risk? Regul Toxicol Pharmacol 54:72-83.

van Ravenzwaay B, Landsiedel R, Fabian E, Burkhardt S, Strauss V, Ma-Hock L. 2009. Comparing fate and effects of three particles of different surface properties: nano-TiO(2), pigmentary TiO(2) and quartz. Toxicol Lett 186:152-159.

Wallace WE, Keane MJ, Murray DK, Chisholm WP, Maynard AD, Ong T-m. 2006. Phospholipid lung surfactant and nanoparticle surface toxicity: Lessons from diesel soots and silicate dusts. Journal of Nanoparticle Research 9:23-28.

Warheit DB, Webb TR, Colvin VL, Reed KL, Sayes CM. 2007. Pulmonary bioassay studies with nanoscale and fine-quartz particles in rats: toxicity is not dependent upon particle size but on surface characteristics. Toxicol Sci 95:270-280.

Wiemann M, Erlinghagen C, Bruch J, Rehn B. 2010. Adsorption of Lung Surfactant by Particles Studied in an ex vivo Model: Effects of Quartz and Amorphous Silica. Materialwissenschaft und Werkstofftechnik / Special issue: Biomaterials 41:1086-1092.

Critical Experimental Conditions for Testing Combustion Generated Nanoparticles

J. O. BRUCH, IHA (University Clinics Essen); IBE GmbH Marl, Germany

Valuable contribution by
IBE-R&D Institute for Lung Health Principle Researcher: M. Wiemann
NanoCare Consortium (inter alia: BASF, R. Landsiedel; Evonic N. Krüger; Bayer Material Sciences, J. Pauluhn; H. F. Krug, KIT-Karlsruhe; IUTA , T. Kuhlbusch)
NanoGem Consortium
FAT/VDA: studies on DPM toxic effects: thresholds; genotoxicity

Overview of the presentation

- Particle surface and interaction with lung surfactant lining fluid (LSF)
- specific implication of the high surface of NP with LSF
- Dose question
- Selection of discriminant parameters
- Combustion Generated NP (CGNP) - possible effects of chemical compounds at sites of initial deposition; exemplified on Diesel Emission Particle – DEP-

The over-all-limitation

- All studies are multi-dose related (in vitro & in vivo)
- tox studies in the non-overload domain (experimentally determined) : mass metric ≤120 µg/10^6 AM; volume metric appr. 60-120 pl/AM
- The non-overload domain has a very good accordance with human exposure scenarios:
- Realistic human exposure estimates
  - human deposition & animal by the „Multiple-Path Particle Dosimetry Model (MPPD2)“
    with reference to
  - lung surface (HEC) or
  - AM Pool (Pauluhn & DFG MAK-Commission) as common denominator

An important restriction in all tox studies

- In particle tox studies: Load levels such as no effect levels (NELS) or lowest adverse effects levels (LOAEL) are taken as a risk measure

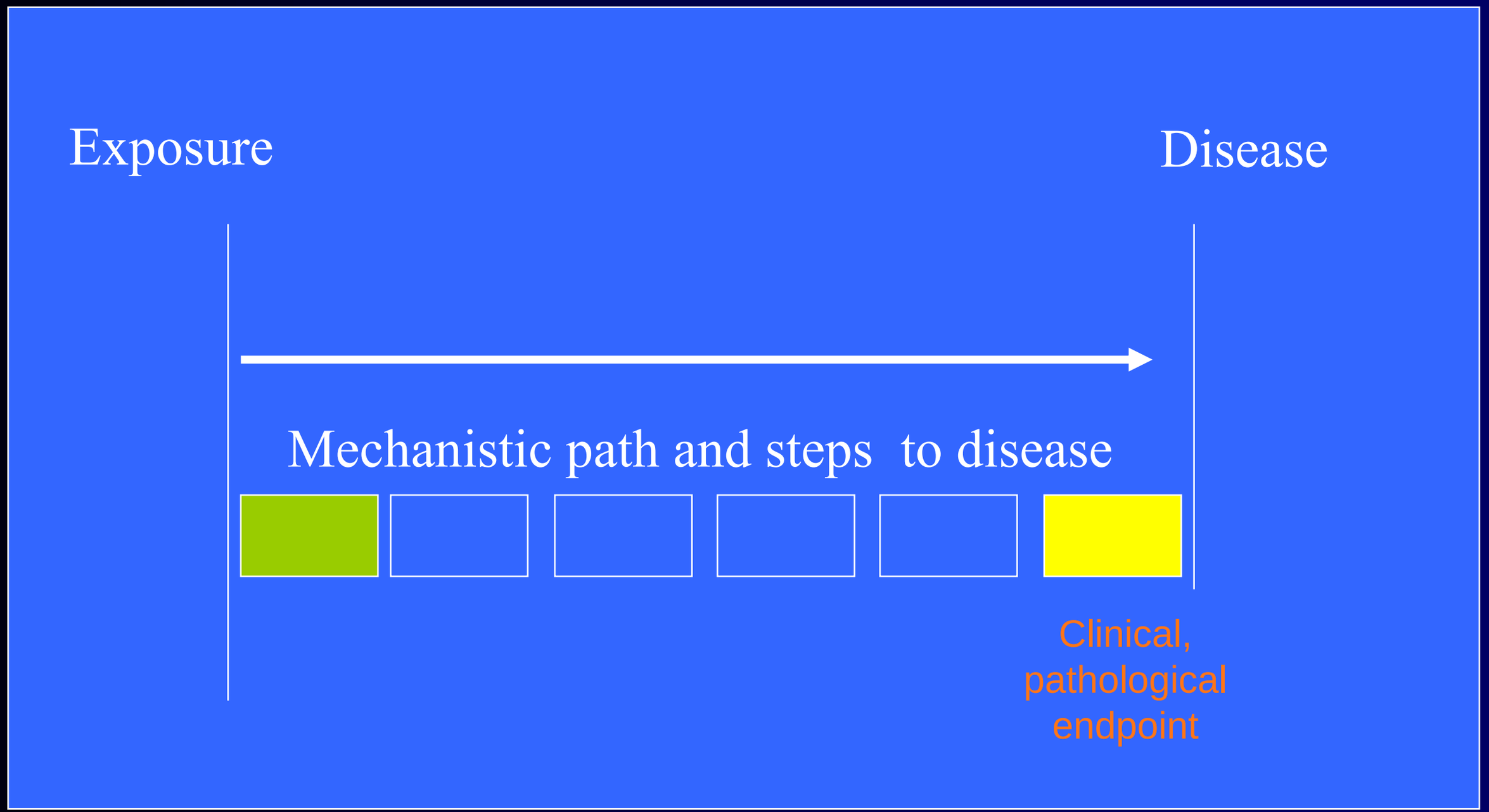
- Dose rate to a target tissue; uptake over the time is critical

- This issue is even more complex in studies for NP or CGNP since the disposal (as dose rate) to the target tissue is dependent from circumstantial conditions; eg. balanced salt medium (MEM) vs. medium containing fetal calf serum (FCS)

The linear sequence of toxic events representation

- Exposure
- inner dose; effect parameters; critical effects; intermediate endpoints; pathology disease
- Figuring out the sequence of upstream & downstream parameters

Path of toxic dust effects



Path of toxic dust effects

Exposure

Disease

Mechanistic path and steps to disease

PSP ENP	LSF Particle Surf.	AM and Particle Incorporation	Inflammation	Interim-Endpoints	

Clinical, pathological endpoint

Initial Phase of deposition of NP at the inner lung surface

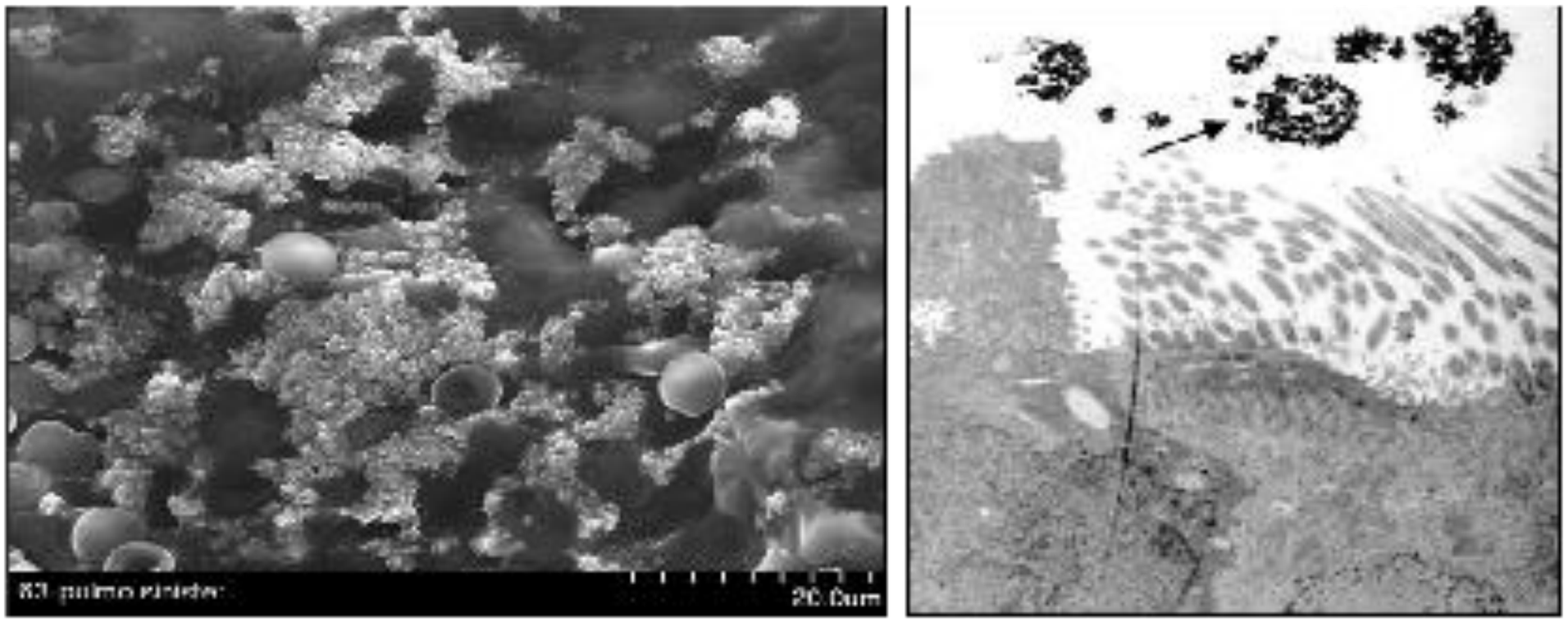


Figure 4.19 TiO_2 (1.2) in the lung by 5-day inhalation study. TiO_2 on the lung surface, SEM (left), TiO_2 agglomerates of various sizes on the lung surface, TEM (right).

NanoCare Final Report; BASF laboratories; R. Landsiedel

Uptake of the NP by the AM

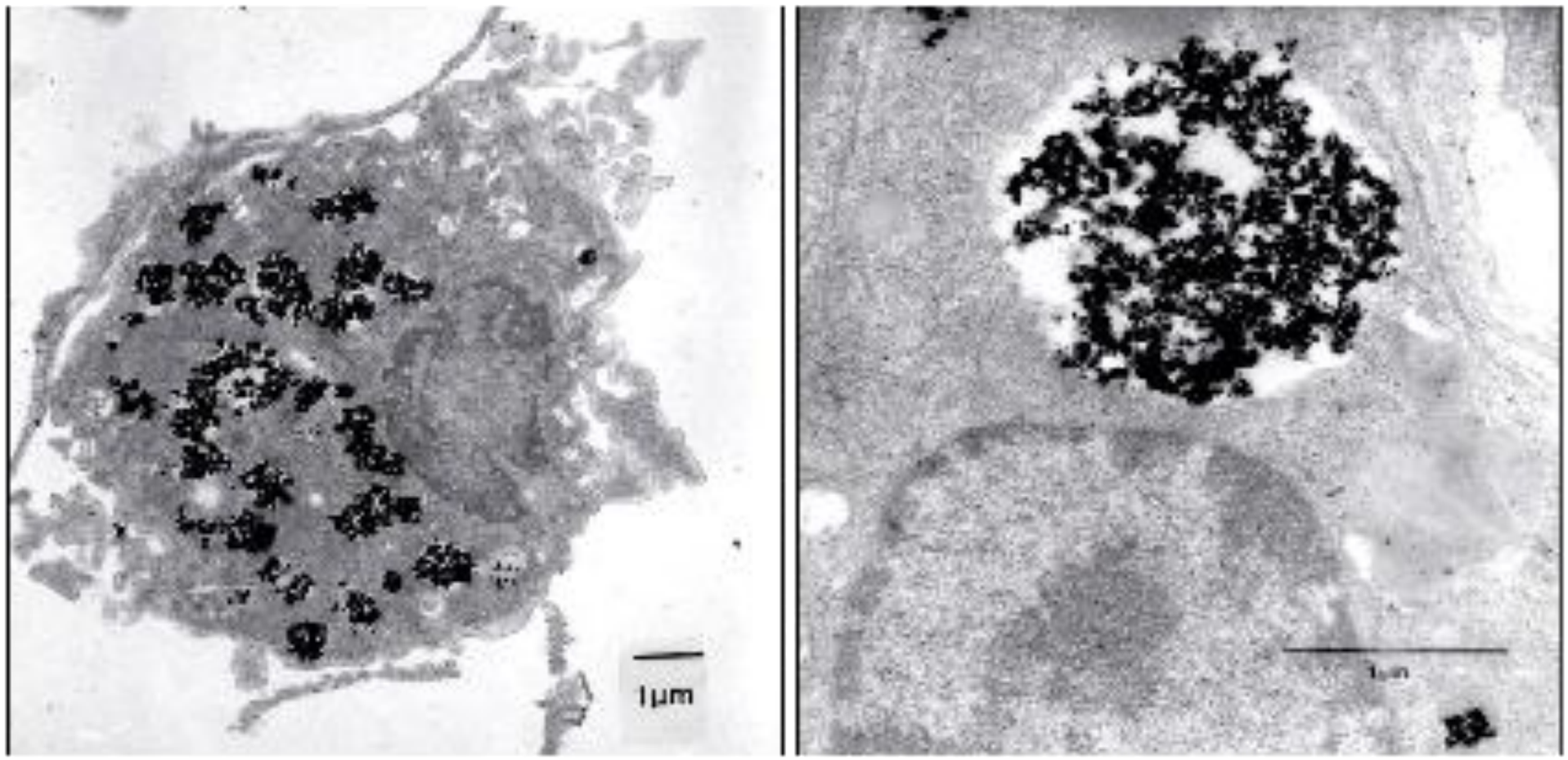
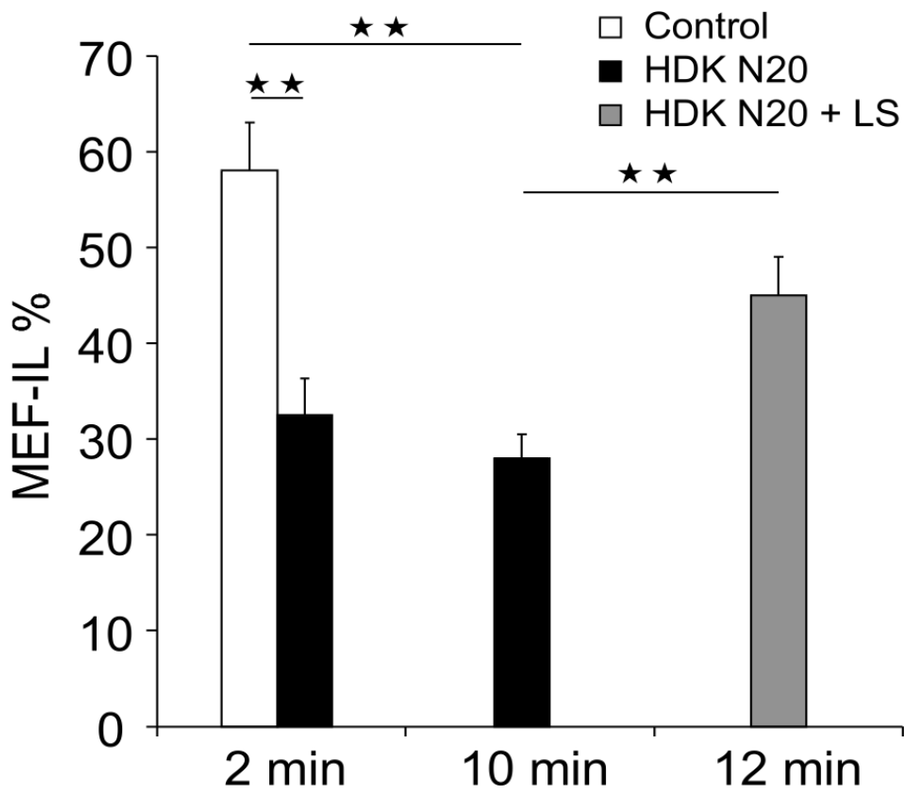


Figure 4.20 TiO_2 (1.2) in the lung by 5-day inhalation study. TiO_2 agglomerates of various sizes in a macrophage in the lung, TEM (left), enlarges picture of an agglomerate of TiO_2 in a macrophage, TEM (right).

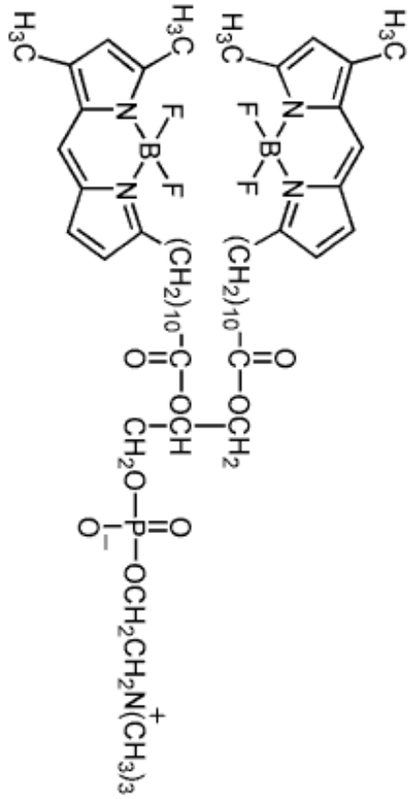
NanoCare Final Report; BASF laboratories; R Landsiedel



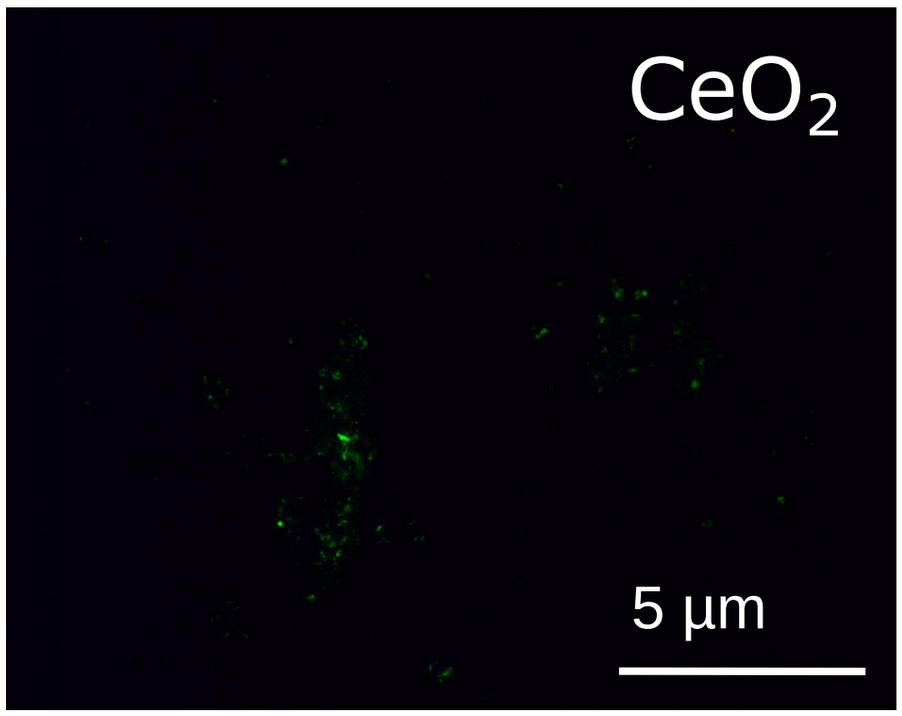
The method introduced in this paper showed that the amorphous silica HDK N20 impaired MEF-IL 3-5 fold more than quartz DQ12. This value, however, is about one order of magnitude less than expected from a comparison of the BET surfaces of HDK N20 and quartz DQ12, suggesting that BET surface overestimates the biological relevant surface in terms of surfactant binding, at least under lung conditions

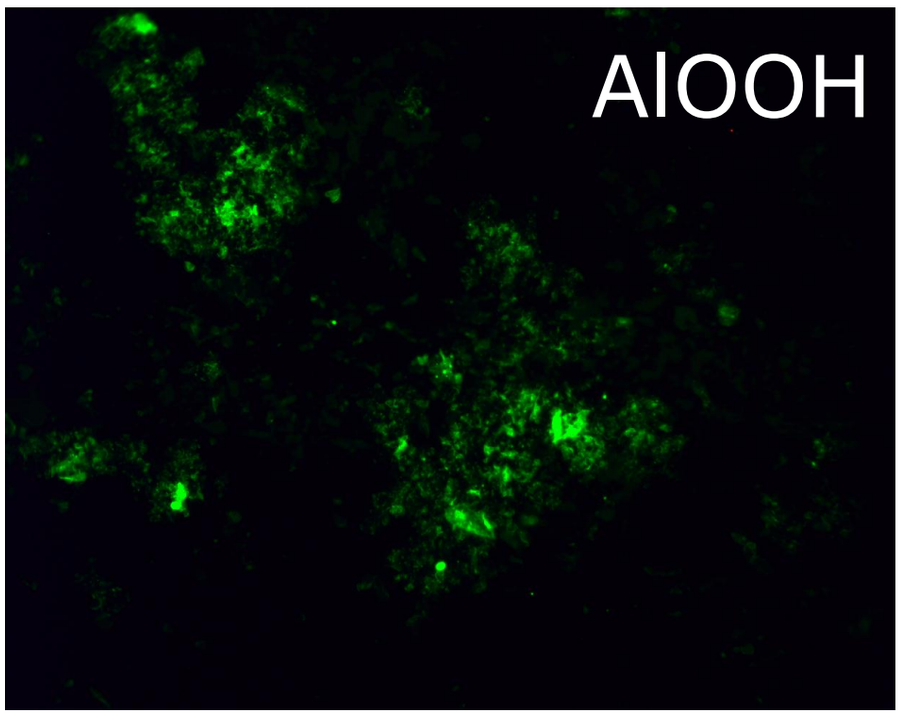
Binding of BOPIPY-Phosphatidylcholine to Nanoparticles

primary particle size:40-50 nm
agglomerates viewed with epifluorescence (Ex.490/Em.535nm)



BODIPY-PC





primary deposition; possible interaction with LSF

Interaction of the LSF with the particle (NP, CGNP) surface

- Agglomeration de-agglommeration of NP
- adsorption; selective a.; depletion of a critical biomolecules from the LSF; denaturation of adsorbed proteins: potential effects of the bio-functional integrity of the alveolar environment
- desorption from the NP-Surface eg. PAH from CGNP
- experimental design: the presence of LSF is in in vitro-studies

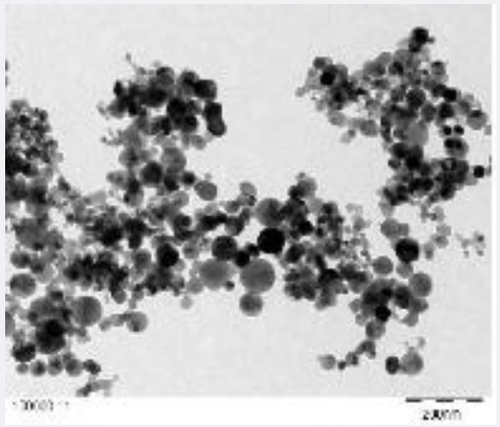
Mutual interation of LSF, Alv. Macrophages and Pneumocytes type II

AM

LSF

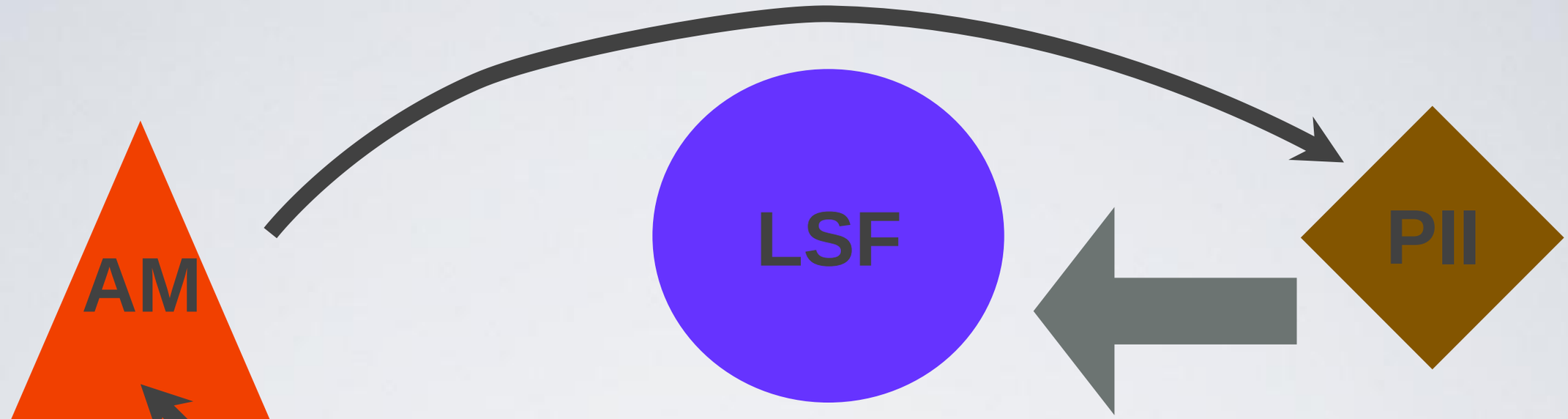
PII

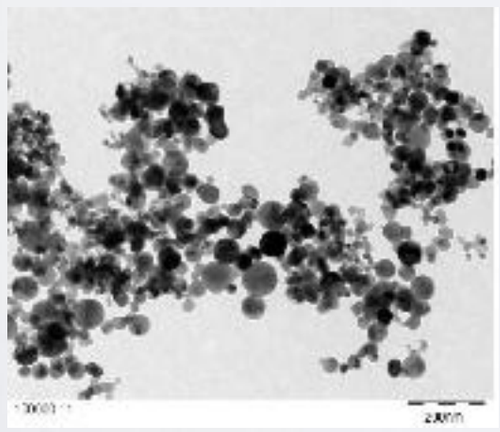
LSF adsorbs
at the particles surface
general or selective
depletion of LSF
constituents;
change of lung function
(compliance)



Adsorption at the
Particle Surface
modifies the particle
bio-activity on AM

Mutual interation of LSF, Alv. Macrophages and Pneumocytes type II





After incorporation of toxic particles, AM release mediators which affect the function of the type II cells and change the composition of the LSF
Clinical and experimental evidence

Important issue *for in vitro* assays

Special focus: Nanoparticles and Occupational Health (2006)

Phospholipid lung surfactant and nanoparticle surface toxicity: Lessons from diesel soots and silicate dusts

William E. Wallace[1,2,*], Michael J. Keane[1], David K. Murray[1], William P. Chisholm[1], Andrew D. Maynard[3] and Tong-man Ong[1]

- Research on surfactant and serum interactions with respirable particle surfaces has indicated profound effects on the expression of toxicity suggesting that interactions of respired NP with biological molecular constituents of the hypophase liquid lining of the lung should be considered in the preparation and interpretation of bioassays of potential NP respiratory hazard.

Path of toxic dust effects

Exposure

Disease

Mechanistic path and steps to disease

PSP

LSF
Particle Surface

AM and Particle
Incorporation

Inflammation

Intermediate
Endpoints

Clinical,
pathological
endpoint

in vitro surrogat tests with permanent cell lines (eg. A549)
culture conditions
necessity of using serum (FCS) media

discriminant params
Assays for critical effects

- Current paradigms
  - upstream params
    - inflammation: PMN; inflammatory mediators
  - downstream params
    - oxidative stress: adducts for OS: 8oxoGua.
- additional effects
  - membrane leakage, interstitial inflammation, changed function of pneumocytes II

Path of toxic dust effects

Exposure

Disease

Mechanistic path and steps to disease

PSP

LSF
Particle Surface

AM and Particle
Incorporation

Inflammation

Intermediate
Endpoints

Clinical, pathological endpoint

BAL:
PMN
TNF alpha
Ep.Type II
proteine leakage

DNA adducts:
8-oxoGua
Etheno Adducts
interst-lymph. penetration
interstial fibrosis
proteine leakage

Path of toxic dust effects (2)

Exposure

Mechanistic path and steps to disease
Additional new effect mechanisms by disposal of chemicals

Disease

Mobilzation of org. compounds by the LSF
lateral diffusion of lipophylic compounds

additional pathogenic vectors

Chem. Comp.

PSP

LSF
Particle Surface

AM and Particle Incorporation

Inflammation

Interim Endpoints

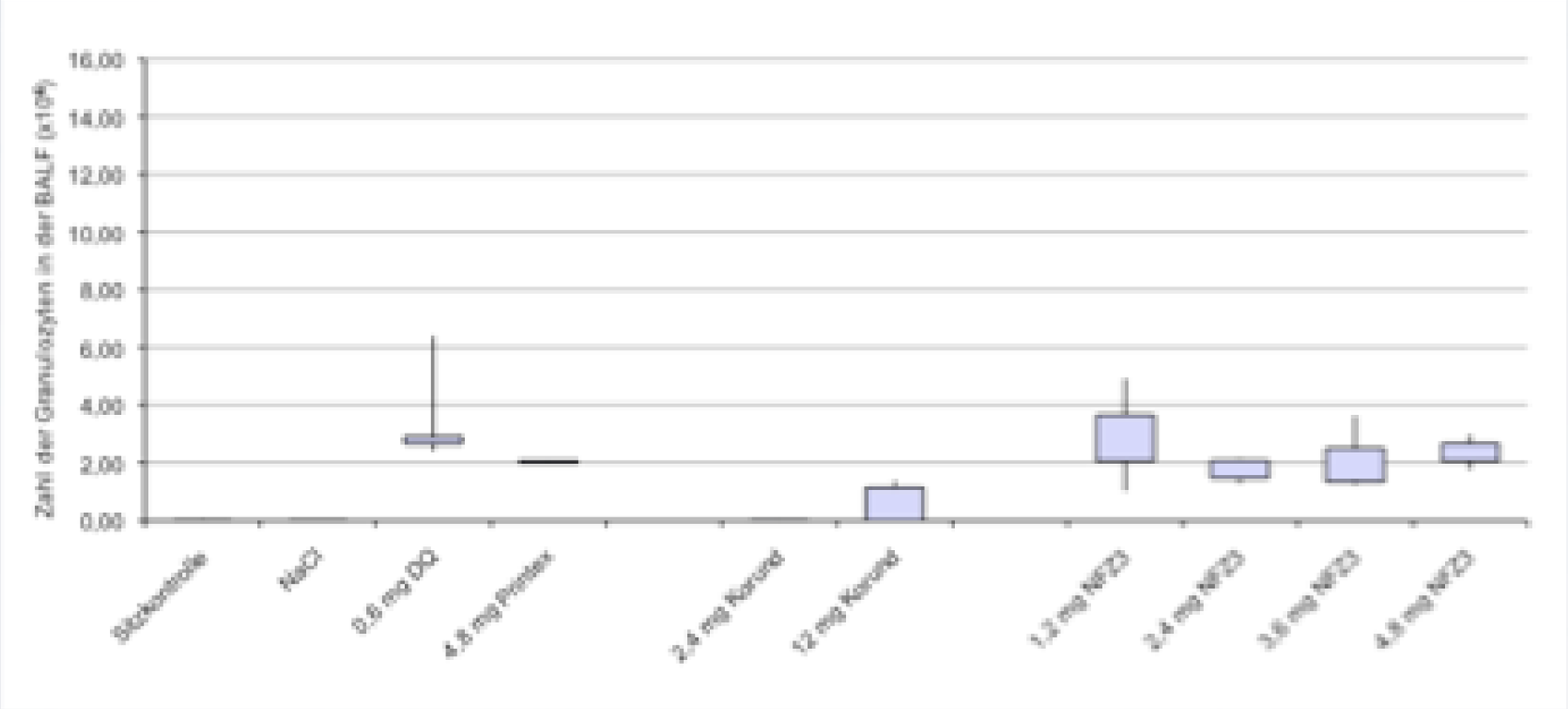
BAL:
PMN
TNF alpha
pneum. type II
proteine leakage

DNA adducts:
8-oxoGua
Etheno Adducts
interst-lymph. penetration
interstial fibrosis
proteine leakage

Clinical, pathological endpoint

Data from the research report for the FATeffects of DEPs heavy duty vs. passenger car

Effects on downstream parameter for genotoxic endpoints (8.oxoGua; p53 mut) in a multi-dose approach

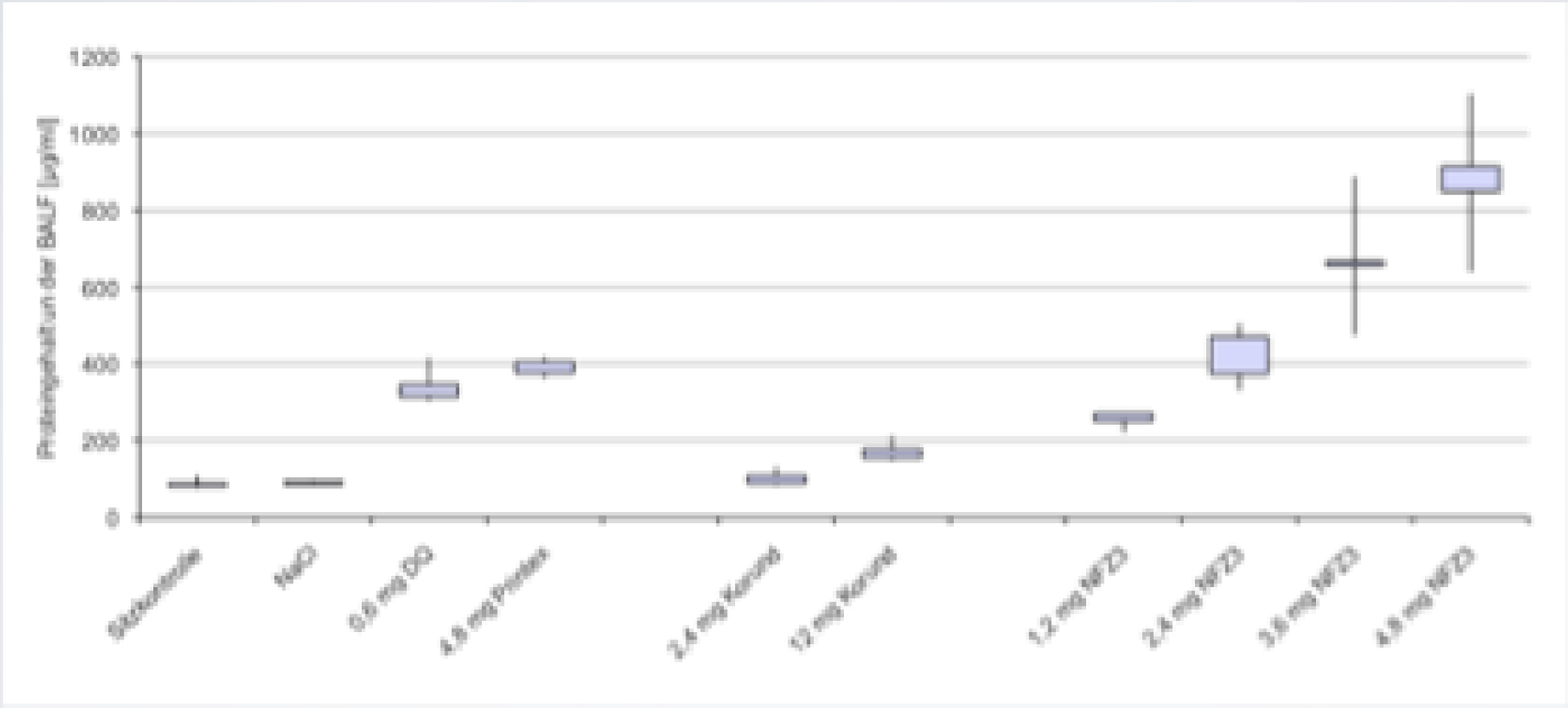


Three day after exposure of a DEP NFZ3: 1.2;. 2.4; 3.6 and 4.8 mg rat lung

no increase of number of PMN over dose in the BAL: toxic effects of DEP on AM; suppression typical inflammatory response

Data from the research report for the FAT effects of DEPs heavy duty vs. passenger car

steep increase of proteine in the BAL with increasing doses of the DPM NFZ3 (heavy duty vehicle) 3 days p. exp.



Three day after exposure of a DEP NFZ3: 1.2;. 2.4; 3.6 and 4.8 mg rat lung toxic effects of DEP on epithelial lining by by soluble chemists (PAH?, Zn?)

Approaching to quantitative risk assessment QRE for CGNP

- Testing in the non-overload domain
- multi dose (dose doubling manner) and multi time test pattern
- identification of the NEL, determination of the zero equivalent dose: ZED
- Comparing the ZEDs with reference substances (eg. Carbon Black
- extrapolation to human exposure with RIVM and AM-pool as common denominator (as recently proposed Pauluhn 2011)