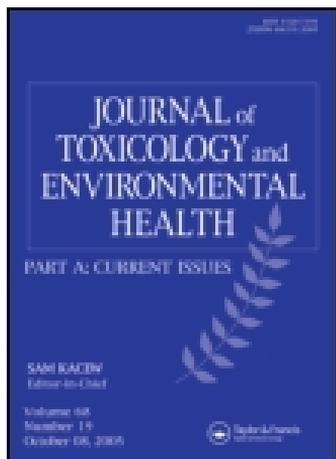


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### EXTRAPULMONARY TRANSLOCATION OF ULTRAFINE CARBON PARTICLES FOLLOWING WHOLE-BODY INHALATION EXPOSURE OF RATS

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## EXTRAPULMONARY TRANSLOCATION OF ULTRAFINE CARBON PARTICLES FOLLOWING WHOLE-BODY INHALATION EXPOSURE OF RATS

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*Studies with intravenously injected ultrafine particles have shown that the liver is the major organ of their uptake from the blood circulation. Measuring translocation of inhaled ultrafine particles to extrapulmonary organs via the blood compartment is hampered by methodological difficulties (i.e., label may come off, partial solubilization) and analytical limitations (measurement of very small amounts). The objective of our pilot study was to determine whether ultrafine elemental carbon particles translocate to the liver and other extrapulmonary organs following inhalation as singlet particles by rats. We generated ultrafine  $^{13}\text{C}$  particles as an aerosol with count median diameters (CMDs) of 20–29 nm (GSD 1.7) using electric spark discharge of  $^{13}\text{C}$  graphite electrodes in argon. Nine Fischer 344 rats were exposed to these particles for 6 h. in whole-body inhalation chambers at concentrations of 180 and 80  $\mu\text{g}/\text{m}^3$ ; 3 animals each were killed at 0.5, 18, and 24 h post-exposure. Six unexposed rats served as controls. Lung lobes, liver, heart, brain, olfactory bulb, and kidney were excised, homogenized, and freeze-dried for analysis of the added  $^{13}\text{C}$  by isotope ratio mass spectrometry. Organic  $^{13}\text{C}$  was not detected in the  $^{13}\text{C}$  particles. The  $^{13}\text{C}$  retained in the lung at 0.5 h postexposure was about 70% less than predicted by rat deposition models for ultrafine particles, and did not change significantly during the 24-h postexposure period. Normalized to exposure concentration, the added  $^{13}\text{C}$  per gram of lung on average in the postexposure period was  $\sim 9$  ng/g organ/ $\mu\text{g}/\text{m}^3$ . Significant amounts of  $^{13}\text{C}$  had accumulated in the liver by 0.5 h postinhalation only at the high expo-*

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sure concentration, whereas by 18 and 24 h postexposure the  $^{13}\text{C}$  amount of the livers of all exposed rats was about fivefold greater than the  $^{13}\text{C}$  burden retained in the lung. No significant increase in  $^{13}\text{C}$  was detected in the other organs which were examined. These results demonstrate effective translocation of ultrafine elemental carbon particles to the liver by 1 d after inhalation exposure. Translocation pathways include direct input into the blood compartment from ultrafine carbon particles deposited throughout the respiratory tract. However, since predictive particle deposition models indicate that respiratory tract deposits alone may not fully account for the hepatic  $^{13}\text{C}$  burden, input from ultrafine particles present in the GI tract needs to be considered as well. Such translocation to blood and extrapulmonary tissues may well be different between ultrafine carbon and other insoluble (metal) ultrafine particles.

Ultrafine particles (<10–100 nm) in urban air are mainly derived from anthropogenic sources of mobile and stationary combustion processes. Levels are generally between 1 and 2  $\mu\text{g}/\text{m}^3$ , or  $1\text{--}2 \times 10^4$  particles/ $\text{cm}^3$  (Hughes et al., 1998). However, during episodic increases mass concentrations can rise to 20–50  $\mu\text{g}/\text{m}^3$  and number concentrations to levels of  $0.3\text{--}1 \times 10^6$  particles/ $\text{cm}^3$  (Brand et al., 1992; Woo et al., 2001). The hypothesis that urban ultrafine particles can cause adverse health effects and contribute to findings in epidemiological studies of increased pulmonary and cardiovascular responses in susceptible parts of the population requires research in different areas. These include evaluation of the composition of single ultrafine particles with respect to their elemental and organic carbon content, as well as nitrates, sulfates, metals, and other constituents; evaluation of the potential for these particles to induce respiratory and cardiovascular effects and their specific underlying mechanisms; and studying the deposition of these particles throughout the respiratory tract and their fate in the organism after deposition. The chemical nature of ultrafine atmospheric particles can change significantly with location and time, which makes it difficult to define a generic ultrafine particle for use in toxicological/clinical studies. However, deposition of inhaled particles in the respiratory tract is largely independent of chemical composition—except for hygroscopic particles—and both the deposition and the fate of the inhaled particles after their deposition are important determinants of potential effects. Since both elemental and organic carbon are significant components of urban ultrafine particles (Hughes et al., 1998; Kittelson, 1998), we suggest that ultrafine elemental carbon particles generated in the laboratory are appropriate surrogate particles for studying their dosimetry (deposition and disposition) following inhalation.

Previous attempts to determine the dosimetry of ultrafine particles have included both theoretical deposition models (ICRP, 1994; NCRP, 1996; Asgharian et al., 1999) and a few laboratory studies measuring deposition and clearance of inhaled ultrafine particles in experimental animals. For example, Kanapilly et al. (1980) exposed rats nose-only to ultrafine  $^{239}\text{PuO}_2$  (10–30 nm) aggregates and found that their deposition in the deep lung was 2–4 times greater than that for  $\mu\text{m}$ -sized particles. They also observed that 47% of the radioactive ultrafine particles were in the gastrointestinal (GI)

tract 2 h postexposure. They did, however, not find any significant translocation to extrapulmonary organs. Biozzi et al. (1953) showed that ultrafine carbon particles (25 nm) administered directly into the blood circulation by intravenous injection accumulated in liver (about 85%) and in spleen (about 15%) of rats, and only when higher doses were injected (>16 mg) were some particles also found in the lungs. In newer studies, translocation of ultrafine metal particles (platinum, silver) to the liver were reported after inhalation by rats (Oberdörster et al., 2000; Finch et al., 1999; Takenaka et al., 2001). However, it cannot be excluded that in vivo dissolution and transport of the dissolved metals were significant factors for these findings. Most recently, Nemmar et al. (2001) exposed human subjects by inhalation to  $^{99m}\text{Tc}$ -labeled ultrafine carbon particles (Technegas) and observed translocation of  $^{99m}\text{Tc}$  into the blood compartment. It is uncertain in this latter study to what degree leaching of the label affected the result due to the difficulty in differentiating quantitatively between the solute label and labelled ultrafine carbon particles in blood and extrapulmonary organs.

In order to overcome the difficulties associated with the use of soluble metal particles or of radioactively-labeled carbon particles, we developed a method to generate ultrafine elemental carbon particles consisting of the stable isotope  $^{13}\text{C}$ . Because of the insoluble nature of elemental carbon (CRC, 2000), these particles should be ideal for examining their fate in the body following deposition by inhalation in the respiratory tract. However, since the natural abundance of  $^{13}\text{C}$  is about 1.1%, the sensitivity of detecting added  $^{13}\text{C}$  in organs by isotope ratio mass spectrometry needed to be determined before applying this method in subsequent studies. We tested the sensitivity of this method in mice by exposing one mouse each to ultrafine  $^{13}\text{C}$  particles (25 nm count median diameter [CMD],  $\sim 100 \mu\text{g}/\text{m}^3$ ) for 30 min or 6 h. The added lung burden of  $^{13}\text{C}$  after 30 min of exposure was determined to be 82 ng, and the added  $^{13}\text{C}$  burden after 6 h of exposure was 199 ng. Using a predictive lung deposition model, this translates into 64% of the inhaled particles being deposited in the lower respiratory tract after 30 min, and only 13% being deposited/retained after 6 h of exposure; that is, a 12-fold longer exposure duration resulted only in a 2.4-fold greater retention of the ultrafine particles in the lung. This result indicated that the sensitivity is sufficient to detect small amounts of added  $^{13}\text{C}$  in tissues; the result also seemed to indicate a significant clearance or translocation of the deposited particles out of the lung during the 6 h of exposure. Thus, the following pilot study was designed to evaluate the deposition and short-term (up to 24 h) fate of ultrafine  $^{13}\text{C}$  particles inhaled by rats.

## METHODS

### Generation of Particles

Ultrafine  $^{13}\text{C}$  particles were generated from pure  $^{13}\text{C}$  graphite electrodes placed in an electric spark discharge generator supplied with argon in the

discharge chamber (Palas Soot Generator, Karlsruhe, Germany). The  $^{13}\text{C}$  graphite electrodes were made by extruding a slurry of amorphous  $^{13}\text{C}$  powder (Isotec, Inc., Miamisburg, OH) mixed with [ $^{13}\text{C}_6$ ]glucose (Isotec, Inc., Miamisburg, OH) through a syringe to produce 3.5-mm-diameter cylinders. These were baked in an argon atmosphere by slowly ramping up the temperature to 200°C within 1.5 h in order to degas the extrusion and decompose the glucose. The electrodes were subsequently graphitized at 2400°C in argon.

### $^{13}\text{C}$ Measurement

Isotopic measurements were performed by continuous-flow isotope-ratio mass spectrometry (Brand, 1996), using a Carlo Erba elemental analyzer coupled to a Finnigan Mat Delta Plus mass spectrometer. The results are reported using the conventional  $\delta$  notation, relative to the PDB standard (fossilized calcite standard), expressed as per mille change:

$$\delta^{13}\text{C} = \frac{{}^{13}\text{C}/{}^{12}\text{C}_{\text{sample}} - {}^{13}\text{C}/{}^{12}\text{C}_{\text{standard}}}{{}^{13}\text{C}/{}^{12}\text{C}_{\text{standard}}} \times 1000 \quad (1)$$

where  ${}^{13}\text{C}/{}^{12}\text{C}_{\text{standard}}$  is the ratio of the reference material (PDB) and  ${}^{13}\text{C}/{}^{12}\text{C}_{\text{sample}}$  is the ratio of the sample. Reproducibility was better than 0.1% for both standards and samples, which translates into a detection limit of about 1 ppm of added  $^{13}\text{C}$  (the  ${}^{13}\text{C}/{}^{12}\text{C}$  ratio for PDB is 0.0112372). Since the tissue has a lower  ${}^{13}\text{C}/{}^{12}\text{C}$  ratio than the reference sample, the  $\delta^{13}\text{C}$  value is negative.

### Measurement of Organic $^{13}\text{C}$

In our earlier studies evaluating pulmonary effects of inhaled ultrafine carbon particles, we had observed that using pure normal graphite electrodes in the Palas soot generator resulted in a significant fraction (20–30%) of organic material associated with the ultrafine carbon fraction. This organic material may be soluble *in vivo*. The source of this is unknown, but is likely due to contaminating organics coming from plastic parts inside the generator rather than from miniscule impurities of the graphite electrodes. In order to evaluate whether any  $^{13}\text{C}$ -rich organic matter was produced during the generation process from our  $^{13}\text{C}$  electrodes, the following test was undertaken. The ultrafine  $^{13}\text{C}$  particles were collected on quartz glass filters and weighed. The filters were placed in a 6-mm OD silica tube in a helium stream, with a heated copper oxide furnace “downstream” from the sample. Samples were step heated to 1000°C using an external resistance heater monitored by a thermocouple placed adjacent to the sample on the outside of the quartz tube. During the heating, any organic matter present will volatilize. The evolved gas is carried along to and oxidized in the copper oxide furnace and then collected in a liquid nitrogen trap further down the He flow path. Gas yields and their  $\delta^{13}\text{C}$  values were determined using a continuous-flow mass spectrometer (Finnigan MAT Delta XL Plus). If  $^{13}\text{C}$  genera-

tion had produced organic matter, then the  $\delta^{13}\text{C}$  values of  $\text{CO}_2$  measured in the mass spectrometer would be extremely high.

### Animal Exposure

Male Fischer 344 rats at a body weight of 250 to 260 g were used for the study. The exposure was for 6 h in compartmentalized whole-body exposure chambers and was performed in 2 sessions. Three rats were exposed in the first session at a concentration of  $180 \mu\text{g}$  ultrafine  $^{13}\text{C}$  particles/ $\text{m}^3$  (CMD = 29.7 nm; GSD [geometric standard deviation] = 1.7). Six rats were exposed in the second session at a concentration of  $80 \mu\text{g}$  ultrafine  $^{13}\text{C}$  particles/ $\text{m}^3$  (CMD = 22 nm; GSD = 1.8). Six unexposed rats served as controls.

### Organ Preparation and $^{13}\text{C}$ Analysis

At 0.5, 18, and 24 h postexposure, rats from each session were sacrificed and lung, liver, heart, olfactory bulb, brain, and kidney were removed for analysis. The excised organs were weighed. Great care was taken to avoid contamination of the organs with  $^{13}\text{C}$  that was deposited on the fur of the animals. To achieve this, the animals were killed by an overdose of intraperitoneal pentobarbital and the fur was wet-wiped with clean tissues. Subsequently the animals were completely skinned and the carcasses rinsed under water. The animals were then moved to a separate room and organs were dissected there with different clean sets of instruments. Each of the organs was homogenized in different clean homogenizers and subsequently lyophilized. Two 1-mg samples of each organ were used for  $\delta^{13}\text{C}/^{12}\text{C}$  isotope ratio determinations using continuous-flow mass spectroscopy for 3–6 replicate measurements. Results were subjected to a weighted two-way analysis of variance (ANOVA), with significant increases in  $^{13}\text{C}/^{12}\text{C}$  ratios being defined as when  $p < .05$ . The two factors in the ANOVA were exposure group and time. The observations were means of replicate measurements, and the number of replications was used as the weight.

The difference in  $\delta^{13}\text{C}$  between organs of exposed rats and the average of respective organs of control rats was used to calculate the added  $^{13}\text{C}$  organ burden. In order to translate  $\delta^{13}\text{C}$  values into absolute amounts of  $^{13}\text{C}$  added to each organ, the baseline carbon content—that is, the sum of  $^{12}\text{C}$  and  $^{13}\text{C}$ —of the different organs needs to be known. Such baseline levels are summarized for humans as Reference Man values by ICRP (1992). For example, lung total carbon content is 10% of lung weight and liver total carbon content is 14.4% of liver weight. Assuming that the carbon content of organs in rats and humans is the same or very similar, the baseline  $^{13}\text{C}$  can be determined using the Reference Man data. These baseline values were used to quantify the added  $^{13}\text{C}$  in exposed compared to control animals. Since the exposure concentrations of the two sessions were very different, that is, 180 and  $80 \mu\text{g}/\text{m}^3$ , the result of the  $^{13}\text{C}$  organ content was normalized to the exposure concentration and expressed two ways, as either added  $^{13}\text{C}/\text{g}$  organ/ $\mu\text{g}/\text{m}^3$  or  $\text{ng } ^{13}\text{C}/\text{total organ}/\mu\text{g}/\text{m}^3$ .

## RESULTS

Step heating of the filter samples with the ultrafine  $^{13}\text{C}$  particles to  $1000^\circ\text{C}$  showed that almost no organic matter was present. The delta values were not elevated above normal, indicating that the  $^{13}\text{C}$  generation process does not produce organic  $^{13}\text{C}$  compounds and that any elevated  $^{13}\text{C}$  found in the organs of the exposed animals is elemental carbon.

Figure 1 shows the results of the  $\delta^{13}\text{C}$  measurements in lungs and livers of rats exposed to the ultrafine  $^{13}\text{C}$  particles for 6 h at 180 and 80  $\mu\text{g}/\text{m}^3$ , respectively. The lungs and also the livers of the exposed animals have increased  $\delta^{13}\text{C}$  values. Lung  $\delta^{13}\text{C}$  was significantly increased at all postexposure times at both exposure concentrations, and lung  $\delta^{13}\text{C}$  did not decrease between 0.5 and 24 h postexposure. For the liver,  $\delta^{13}\text{C}$  was significantly increased already at 0.5 h postexposure at the higher exposure concentration and stayed elevated throughout the 24-h period. In contrast, at the lower exposure concentration, significantly increased  $\delta^{13}\text{C}$  values were observed in the liver only at 18 and 24 h postexposure.

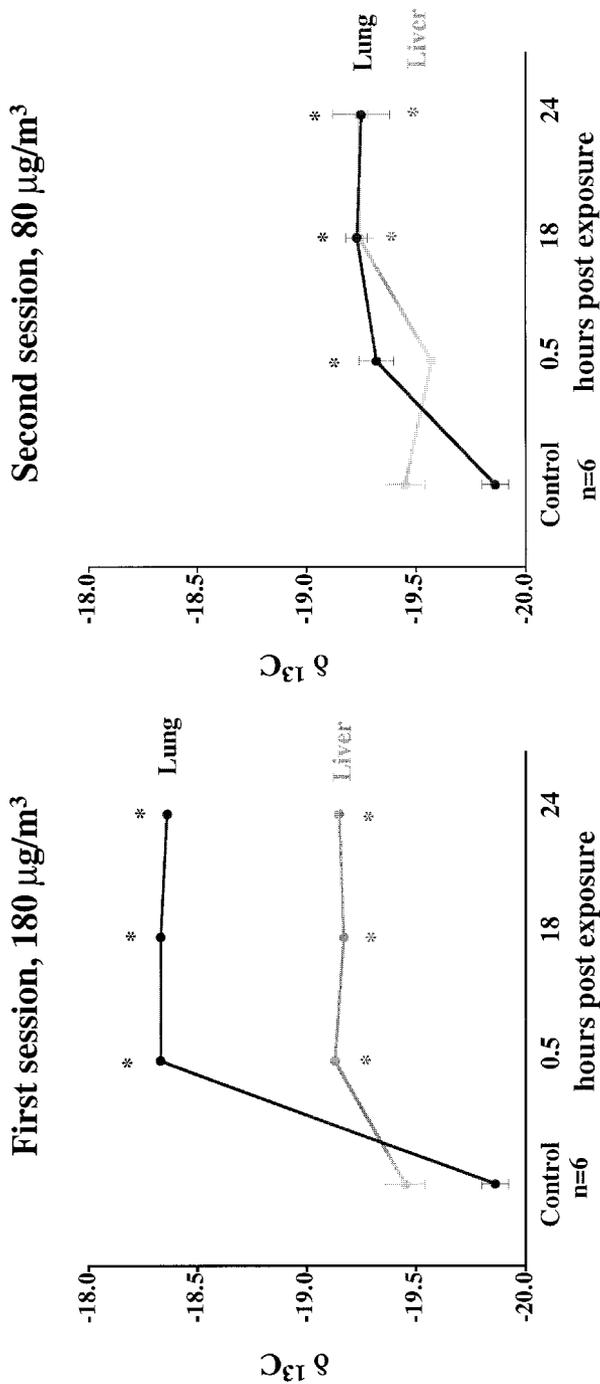
Figure 2 depicts the lung and liver excess  $^{13}\text{C}$  concentration normalized to the exposure concentration expressed as mean  $\pm$  standard deviation of ng  $^{13}\text{C}$  added per gram of organ per microgram per cubic meter of exposure concentration. These combined data of exposure sessions 1 and 2 show that there was no significant change in lung  $^{13}\text{C}$  concentration over the 24-h postexposure time-period and that the liver  $^{13}\text{C}$  concentration was significantly increased at 18 and 24 h postexposure. The concentration (per gram organ) of the added  $^{13}\text{C}$  in the liver at 18 and 24 h is  $\sim 2.5$ -fold less than the  $^{13}\text{C}$  concentration in the lung.

However, because the weight of rat livers is about 12 times greater than that of their lungs, the amount of added  $^{13}\text{C}$  per total organ following the exposure to ultrafine  $^{13}\text{C}$  particles is about fivefold higher in the liver at 18 and 24 h postexposure than in the lung, normalized for the exposure concentration—that is, by 24 h,  $43.39 \pm 11.31$  ng  $^{13}\text{C}/\mu\text{g}/\text{m}^3$  (liver) versus  $8.35 \pm 1.57$  ng  $^{13}\text{C}/\mu\text{g}/\text{m}^3$  (lung). There was no significant decrease in lung  $^{13}\text{C}$  between 0.5 and 24 h postexposure, which means that at 18 and 24 h postexposure more  $^{13}\text{C}$  had accumulated in the liver than was found to be retained in the lung at 0.5 h postexposure (see Table 1). Thus, following 6-h exposure to 180  $\mu\text{g}/\text{m}^3$ , 5.7  $\mu\text{g}$   $^{13}\text{C}$  was added to the liver by 24 h, versus 1.43  $\mu\text{g}$   $^{13}\text{C}$  retained in the lung by 0.5 h postexposure. For the rats exposed to 80  $\mu\text{g}/\text{m}^3$  for 6 h, 3.54  $\mu\text{g}$   $^{13}\text{C}$  and 4.34  $\mu\text{g}$   $^{13}\text{C}$  were added to their livers by 24 h, whereas only 0.62  $\mu\text{g}$   $^{13}\text{C}$  and 0.50  $\mu\text{g}$   $^{13}\text{C}$  were retained in their lungs, respectively. No significant increase in  $\delta^{13}\text{C}$  was found in heart, olfactory bulb, brain, or kidney by 24 h postexposure.

## DISCUSSION

This study showed a significant increase in  $^{13}\text{C}$  content in the liver of rats by 18 and 24 h following a 6-h exposure to ultrafine elemental  $^{13}\text{C}$  particles.

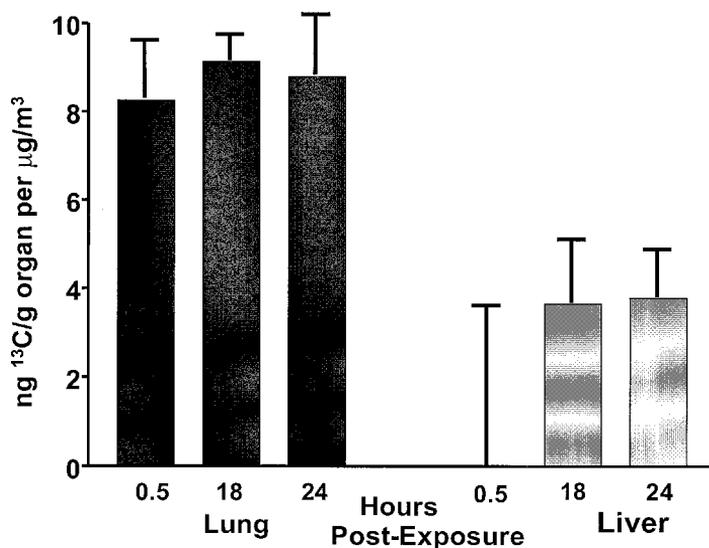
# $\delta^{13}\text{C}$ in Lung and Liver after 6 hr of $^{13}\text{C}$ Inhalation



\* Significant increase, 2-way ANOVA ( $p < 0.05$ )

FIGURE 1.  $\delta^{13}\text{C}$  in lung and liver after 6-h exposure of rats to ultrafine  $^{13}\text{C}$  particles by inhalation.

### Normalized Lung and Liver Excess $^{13}\text{C}$ Concentration Following Ultrafine $^{13}\text{C}$ Particle Exposure in Rats ( $n=3$ )



**FIGURE 2.** Normalized lung and liver excess  $^{13}\text{C}$  concentration following ultrafine  $^{13}\text{C}$  particle exposure in rats for 6 h ( $n = 3$ , mean  $\pm$  standard deviation).

Because elemental carbon particles are insoluble (CRC, 2000), this finding implies the translocation of ultrafine carbon particles following whole-body exposure to the blood circulation with subsequent uptake in the liver. That the liver is the major organ where blood-borne ultrafine particles accumulate is consistent with earlier observations of intravenously injected ultrafine carbon particles depositing preferentially in the liver and to a lesser degree in the spleen (Biozzi et al., 1953). Spleen  $^{13}\text{C}$  analysis was not performed in

**TABLE 1.** Lung and Liver Excess  $^{13}\text{C}$  Following Exposure to Ultrafine  $^{13}\text{C}$  Particles

Exposure concentration ( $\mu\text{g}/\text{m}^3$ )	Time postexposure					
	0.5 h		18 h		24 h	
	Lung ( $\mu\text{g}$ )	Liver ( $\mu\text{g}$ )	Lung ( $\mu\text{g}$ )	Liver ( $\mu\text{g}$ )	Lung ( $\mu\text{g}$ )	Liver ( $\mu\text{g}$ )
180	1.43 (rat 1)	6.33	1.52 (rat 2)	5.44	1.47 (rat 3)	5.70
80	0.62 (rat 4)	0	0.72 (rat 5)	2.70	0.80 (rat 6)	3.54
80	0.50 (rat 7)	0	0.63 (rat 8)	4.62	0.55 (rat 9)	4.34

this pilot study, but will be crucial in future studies to differentiate between different pathways of entry to the blood circulation of inhaled ultrafine particles, that is, pulmonary versus hepatic circulation. No significant change in the lung burden of ultrafine carbon particles during the 24-h postexposure period was observed, and no indication of an increase in  $^{13}\text{C}$  was seen in the other extrapulmonary organs that were examined.

The total added  $^{13}\text{C}$  amount in the liver by 24 h was much greater than the amount retained in the lung, and the question arises as to whether all of the ultrafine carbon particles that translocated to the liver reflect those that initially were deposited somewhere in the respiratory tract. In order to estimate whether some of this translocation may have derived from additional sources, such as ultrafine carbon particles cleared into the GI tract, a multiple-path particle deposition model (MPPDep; Asgharian et al., 1999) was used to predict the deposited amount of ultrafine carbon particles during the 6-h inhalation period in rats. Table 2 shows the result of these model predictions compared to the  $^{13}\text{C}$  burdens found in lung and liver at 0.5 h and 24 h after the exposure. Two sources of uncertainty in Table 2 data have to be considered. One is that although the model predictions of the deposition of inhaled particles in rats are based on well-accepted theoretical models, they have not been confirmed for ultrafine particles through experimental data; the other is related to the accuracy of the total carbon content of lungs and liver in the rat, since our calculations of  $^{13}\text{C}$  organ burden are based on the data of total organ carbon in humans from ICRP Reference Man (1992). However, despite these uncertainties, the main finding of this study does not change: In rats, significant amounts of ultrafine elemental carbon particles translocate to the liver by 18 and 24 h postinhalation.

**TABLE 2.** Model Prediction Versus Recovered  $^{13}\text{C}$  in Lung and Liver Following 6-h Exposure to Ultrafine  $^{13}\text{C}$  Particles

Exposure concentration ( $\mu\text{g}/\text{m}^3$ )	Predicted lung deposition (TB + A) ( $\mu\text{g}$ )	Found in lung, 0.5 h ( $\mu\text{g}$ )	Percent of predicted lung deposition found in lung at 0.5 h	Predicted deposition in total respiratory tract ( $\mu\text{g}$ )	Found in liver + lung at 24 h ( $\mu\text{g}$ )	Percent of predicted total respiratory-tract deposition in liver + lung at 24 h
180	4.03	1.43 (rat 1)	35.5	5.43	7.17 (rat 3)	132
80	1.89	0.62 (rat 4)	32.8	2.59	4.34 (rat 6)	167
80	1.89	0.50 (rat 7)	26.5	2.59	4.89 (rat 9)	189

Thus, although the mentioned uncertainties exist, the evaluation of this study regarding the comparison of predicted versus retained  $^{13}\text{C}$  in Table 2 allows several conclusions to be drawn. One is that the predicted lung deposition in the tracheobronchial and alveolar regions (~30% of the inhaled ultrafine particles based on the MPPDep model) is far greater than the amount of ultrafine carbon found at 0.5 h postexposure in the lower respiratory tract, which has to be interpreted that, on average, about two-thirds of the lung deposits have been cleared during the 6 h of exposure and 30 min postexposure. Since no significant lung clearance occurred between 0.5 and 24 h postexposure, these data imply a rather rapid initial clearance phase of the ultrafine  $^{13}\text{C}$  particles from the lower respiratory tract.

We had observed in our earlier studies with ultrafine polytetrafluoroethylene (PTFE) particles that those depositing in the lower respiratory tract translocate rapidly to epithelial, interstitial and endothelial sites, although we could not quantify the amount of translocation via this pathway (Oberdörster et al., 2000). These particles seem to escape to a large degree phagocytosis by alveolar macrophages (AM), as was suggested by results of these studies. This suggestion is supported by earlier studies (Pratten & Lloyd, 1986) showing a 70-fold slower rate of uptake by macrophages for 30-nm particles compared to 1.1- $\mu\text{m}$  particles. Such uptake by epithelial cells can also be deduced from results by Kreyling et al. (2002), who found that following exposure of rats to ultrafine iridium particles, only 20% or less of the lung burden of these particles was present in the cell pellet from lung lavages at any time point postexposure. This is in agreement with studies by Ferin et al. (1991, 1992), who demonstrated that significantly fewer ultrafine  $\text{TiO}_2$  particles were lavagable from rat lungs, in contrast to about 80–90% of larger particles that were lavageable with the cell pellet. These authors concluded that phagocytosis of particles by AM appears to be the mechanism that prevents the rapid translocation of particles from the alveoli into the pulmonary tissue (Ferin et al., 1991). From interstitial sites in the respiratory tract, further translocation of the ultrafine particles to the blood circulation via lymphatic channels or directly via the endothelium could take place. The fact that liver  $\delta^{13}\text{C}$  was already increased at 0.5 h after exposure to 180  $\mu\text{g}/\text{m}^3$  in the present study speaks for this interstitial clearance pathway into the blood circulation, which may indeed be dose dependent since liver  $\delta^{13}\text{C}$  was not increased at the lower exposure concentration at this early time point.

In addition to the interstitial clearance pathway from the alveolar region, deposits of ultrafine  $^{13}\text{C}$  particles in the tracheobronchial region can be cleared via the mucociliary escalator into the GI tract. Likewise, ultrafine carbon particles deposited in the head region may also, for the most part, be cleared into the GI tract, although it cannot be excluded that clearance into the blood circulation from areas throughout the respiratory tract has occurred. Thus, the contribution of ultrafine particles cleared into the GI tract needs to be considered.

The amount of ultrafine  $^{13}\text{C}$  particles found in lung and liver at 24 h post-exposure is on average 63% greater than what is predicted to be deposited in the total respiratory tract (Table 2). This implies that in addition to inhalation there were other sources of input and pathways of ultrafine  $^{13}\text{C}$  into the organism in this study, a likely source being external contamination of the animals' fur during the 6-h whole-body exposure. Both during the exposure as well as postexposure, rats will clean their fur thoroughly and thereby will take in externally deposited ultrafine carbon particles into the GI tract. It is therefore conceivable that a significant amount of the ultrafine  $^{13}\text{C}$  found in the liver in the postexposure phase is derived from the GI tract, due both to the already mentioned clearance via mucociliary action and to oral intake from skin deposits.

A GI route of translocation of ingested ultrafine particles to the blood, in addition to direct translocation to the blood from the respiratory tract, is supported by studies in rats and humans that have shown that  $\text{TiO}_2$  particles (150–500 nm) taken in via food can translocate to the blood and are taken up by liver and spleen (Jani et al., 1994; Böckmann et al., 2000). Furthermore, earlier studies by Volkheimer (1974) described a mechanism of persorption in epithelial cells of the GI tract by which even larger particles are taken up into lymphatic and blood circulation and translocate to the liver and other organs. In contrast, the aforementioned studies by Kanapilly and Diel (1980) and by Kreyling et al. (2002) with ultrafine radioactive metal particles do not show significant translocation from the GI tract to other organs via the blood circulation; nor do these studies show significant translocation of ultrafine metal particles deposited in the lung to extrapulmonary tissues.

The translocated ultrafine particles measured in the liver in our study can be due to direct input into the blood compartment from both the respiratory and GI tracts, which differs from the findings by Kanapilly and Diel (1980) and Kreyling et al. (2002). These differences could be due to several factors, including different chemistry of ultrafine particles and different methodologies. Kanapilly and Diel (1980) used nose-only exposures, Kreyling et al. (2002) used intratracheal inhalation (thereby circumventing the nose), and in our present study we used whole-body exposures. The amount of ultrafine particles being translocated to the GI tract via physiological clearance processes may have been different between those studies, and it is unknown whether binding of ultrafine particles to cellular components or proteins occurs, which conceivably could be different for metals and carbon. Additional studies with ultrafine carbon particles are planned to evaluate specific translocation pathways into the blood circulation through the upper and lower respiratory tract and through the GI tract.

## CONCLUSIONS AND OUTLOOK

Use of the stable isotope  $^{13}\text{C}$  for generation of ultrafine carbon particles is a sensitive alternative to radioactively labeled particles for determining

the deposition and fate of inhaled insoluble ultrafine particles in the respiratory tract and extrapulmonary organs. There appears to be rapid initial clearance/translocation of ultrafine  $^{13}\text{C}$  particles during an inhalation exposure of rats from the lower respiratory tract. The fact that by 18 and 24 h post  $^{13}\text{C}$  particle exposure the  $^{13}\text{C}$  content of the liver is significantly increased shows that a large amount of the ultrafine carbon particles reached the blood circulation. In addition to direct input into the blood compartment from ultrafine particle deposits throughout the respiratory tract, there could be significant input from the GI tract due to mucociliary clearance, as well as postexposure cleaning of their fur by the whole-body-exposed rats resulting in significant dosing of the GI tract with ultrafine carbon particles. It needs to be determined whether the liver may serve as a storage organ for ultrafine carbon particles, which subsequently may be redistributed to other organs beyond the 24-h postexposure time point. The findings in epidemiological studies of lag-time effects of ambient particles in sensitive parts of the population could be related to such delayed distribution. An apparent difference in the behavior of translocation of ultrafine carbon versus ultrafine insoluble metal particles needs to be evaluated further, especially when considering that ambient ultrafine carbonaceous particles also contain metals such as iron, vanadium, and zinc.

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