Titanium Dioxide Nanoparticles: Grassian et al. Respond.

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Aggregation and Toxicology of Titanium Dioxide Nanoparticles
doi:10.1289/ehp.10915

In their study of inhalation exposure of titanium dioxide particles, Grassian et al. (2007) presented a transmission electron micrograph (TEM) (their Figure 2A) as an image of “dispersed” TiO₂ nanoparticles. Yet, the TiO₂ nanoparticles in this TEM do not appear to be dispersed. There is clear evidence of self-organization of the nanoparticles into distinct assemblages, separated by relatively large regions devoid of any particle. This spatial pattern, very unlikely to occur randomly, is even more apparent when Grassian et al.’s TEM is contrast-enhanced, sharpened, and thresholded (Figure 1A) to eliminate the initial grainy background. With this image, one can demonstrate quantitatively the extent of clustering by calculating the radial distribution function (Torquato 2002), defined as the probability of finding a nanoparticle, in any direction, at various distances away from the center of a given nanoparticle. We compared the values obtained for this function with those associated with an image in which the same nanoparticles have been artificially dispersed (with image processing software). In the dispersed case (Figure 1B), the probability of finding a black pixel drops precipitously when the distance exceeds the apparent radius of nanoparticles, and then stays close to zero thereafter. In the “original” case (Grassian et al.’s Figure 2A), there is also a drop, but the radial distribution function never gets to zero. It progressively increases again as the radial distance increases. This quantitative difference between the curves in Figure 1B leads to the conclusion that the nanoparticles in Figure 1A are clustered.

However, this conclusion is intriguing in itself. Indeed, before obtaining their TEM, Grassian et al. (2007) suspended the TiO₂ nanoparticles in methanol and sonicated the suspension for an unspecified, but presumably appreciable “period of time.” Given this strongly dispersive treatment, it is remarkable that aggregation still occurred to the extent it did. This observation suggests that the 2- to 5-nm size of the primary TiO₂ “nano”-particles may be somewhat irrelevant to environmental and toxicologic concerns because in nature, under conditions far more conducive to aggregation than those imposed by Grassian et al. (2007), nanoparticles may never be found alone, but are part of significantly larger-sized aggregates. In a recent study, French et al. (French RA, Jacobson AR, Kim B, Isley SL, Penn RL, Baveye PC, unpublished data) observed that in aqueous suspensions under a range of environmentally relevant conditions of pH and ionic strength, TiO₂ nanoparticles form aggregates of several hundred nanometers to several micrometers in diameter within minutes.

This aggregation may have toxicologic implications. In any given system (e.g., aerosols), it is possible that even a slight change in pH or ionic strength may cause TiO₂ nanoparticles to cluster differently, and therefore to have very dissimilar biological activity. In general, this might explain mixed results found in the literature on the toxicity of TiO₂ nanoparticles to environmentally relevant species. Until now, these inconclusive results have been explained (Oberdörster et al. 2005) by arguing that the high biological activity of TiO₂ nanoparticles, caused by their large specific surface area, creates a high potential for inflammatory, pro-oxidant, and antioxidant activity. Yet, conflicting observations may perhaps be imputable instead to compounding factors due to nanoparticle aggregation, which so far has not been given serious consideration.

The authors declare they have no competing financial interests.

**References**


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doi:10.1289/ehp.10915R

Baveye and Laba have further analyzed the transmission electron micrograph (TEM) image shown in Figure 2A of our article (Grassian et al. 2007b) to quantitatively determine the extent of titanium dioxide nanoparticle clustering in the image by calculating the radial distribution function. The main point of doing this calculation was to demonstrate that TiO₂ nanoparticle aggregates will not completely deaggregate even when subjected to harsh conditions.

We completely agree with the statement of Baveye and Laba that “aggregation may have toxicologic implications.” We disagree with their suggestion that “nanoparticle aggregation … so far has not been given serious consideration.” There is growing consensus that nanoparticle aggregation is an important factor in understanding the health implications of nanoparticles. This has been described by researchers working in the area of nanoparticle toxicity (Balbus et al. 2007; Powers et al. 2006) as well as by us. In addition to Grassian et al. (2007b), we refer to another study in which we further investigated TiO₂ nanoparticle aggregation in inhalation and instillation studies (Grassian et al. 2007a). In that study we demonstrated that the size and nature of
DDT and Breast Cancer

doi:10.1289/ehp.11025R

In a recent article, Cohn et al. (2007) noted an association between increased breast cancer risk and \( p,p'-\text{DDT} \) [1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane] exposure early in life. Their article should be interpreted with caution, particularly the estimated 5-fold increase in risk for women born after 1931 the authors reported without qualification in the “Abstract”; this value was repeated in the news article by Manuel (2007). Cohn et al. (2007) evaluated three DDT congeners—that is, \( p,p'-\text{DDT} \), \( o,p'-\text{DDT} \) [1,1,1-trichloro-2(p-chlorophenyl)-2(\( o \)-chlorophenyl)ethane], and \( p,p'-\text{DDE} \) [1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene]—by various categories of year of birth, yet they found no significantly increased risk estimates for any of the three DDT congeners in multiple comparisons that were not adjusted for the other DDT-related chemicals either in all women or in women born after 1931. The estimated 5-fold increase in risk for the upper tertile of \( p,p'-\text{DDT} \) serum levels was only observed in subgroup analyses that were both restricted to women born after 1931 and adjusted for serum level of \( o,p'-\text{DDT} \). The impact of the adjustment for \( o,p'-\text{DDT} \) on the risk estimate for \( p,p'-\text{DDT} \) is remarkable in view of the low \( o,p'-\text{DDT} \) levels observed (35% were below the limit of detection). A significant inverse association between \( p,p'-\text{DDT} \) level and breast cancer risk, which was interpreted by Cohn et al. in terms of length of time since DDT exposure, became stronger after adjustment for \( p,p'-\text{DDT} \) levels; presumably this does not indicate a protective effect of recent DDT exposure.

In view of the absence of evidence for an association between \( p,p'-\text{DDE} \) levels and breast cancer risk (Lopez-Cervantes et al. 2004), it seems unlikely that DDT exposure increases the risk of breast cancer. Nonetheless, if the effect of DDT exposure early in life on breast cancer risk is large (a possibility suggested by Cohn et al. (2007)), then the decreasing birth cohort trend in breast cancer risk that has been observed for U.S. baby boomers is even more remarkable (Chu et al. 1999; Tarone 2006, 2007; Tarone and Chu 2000). Women born after 1945 would have been exposed to DDT for each of the first 13 years of life, with increasing exposure through the late 1960s (Wolf et al. 2005), but the birth cohort risk of breast cancer showed a marked decrease among U.S. women for over two decades after 1945. DDT exposure would join a list of other breast cancer risk factors predicting increasing breast cancer risk in baby boomers (Tarone 2006); yet the birth cohort risk of breast cancer decreased for women born after 1945. That the hypothesized association between DDT exposure and breast cancer risk has received far more attention than the paradoxical decreasing risk of breast cancer when actually occurred among young U.S. women says much about the priorities and focus of environmental epidemiology. The author declares he has no competing financial interest.

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We thank Tarone for his letter, as it provides an opportunity to elaborate on analytic strategies for the study of DDT associations with breast cancer. One feature of our study (Cohn et al. 2007)—assessment of exposure in blood samples collected during active DDT use in the 1960s—provided a unique opportunity to examine three DDT-related compounds singly and in combination. The three DDT-related compounds studied represent distinct aspects of exposure. \( p,p'-\text{DDT} \) [1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane] is the primary ingredient of commercial grade DDT. \( p,p'-\text{DDE} \) [1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene], the most persistent DDT-related compound, is a metabolite of \( p,p'-\text{DDT} \) that is both made by humans during active exposure, and also ingested directly from food sources where it can be stored for long periods in fat (Morgan and Roan 1975). \( o,p'-\text{DDT} \) [1,1,1-trichloro-2(\( p \)-chlorophenyl)-2(\( o \)-chlorophenyl)ethane] is a low-concentration contaminant of commercial DDT that is eliminated by humans most quickly, making it a marker of recent exposure (Morgan and Roan 1975). Therefore, absolute and relative DDT/DDE isomer levels may represent different windows of exposure (Wolf et al. 2007).

Unlike our investigation, most other breast cancer studies were conducted long after active use of DDT ceased. Thus the preponderance (> 95%) of their exposure was only \( p,p'-\text{DDT} \) [see our Figure 1 and Table 1 (Cohn et al. 2007)]. Hence, our study provides new information. An additional
dimension is that these compounds have been shown to have distinctly different endocrine activity (Kelce et al. 1995), suggesting potential for differential effects on human outcomes. Therefore, we disagree with Tarone’s assertion that the lack of an association between \( p,p' \)-DDE and breast cancer risk in young women refutes a role for \( p,p' \)-DDT exposure. The timing, origin, and functional activity may differ for each compound.

Concurrent measurements of \( p,p' \)-DDT, \( p,p' \)-DDE, and \( o,o' \)-DDT allow evaluation of potential differences in the effects of these compounds. Other studies have also observed differing associations with cancer risk for \( p,p' \)-DDT and its metabolite, \( p,p' \)-DDE. McGlynn et al. (2006) reported that the \( p,p' \)-DDT association with risk of liver cancer was enhanced when \( p,p' \)-DDE was low. We also reported that a higher proportion of \( p,p' \)-DDE to \( p,p' \)-DDT in maternal serum samples was associated with longer time to pregnancy in their daughters 30 years after exposure in utero (Cohn et al. 2003). In another breast cancer study, Romieu et al. (2000) showed a significant effect for \( p,p' \)-DDE—after adjustment for \( p,p' \)-DDT—for predicting breast cancer, particularly in postmenopausal women. We believe that simultaneous adjustment for DDT-related compounds is a strength of our study.

Tarone suggests that subgroup analyses weaken the results of our article (Cohn et al. 2007). However, we pointed out in our article that subgroup analyses, by birth cohort, were planned a priori and were a primary objective of our study. In this setting, subgroup analyses are a strength that enabled us to examine whether age at DDT exposure may be of importance in human breast cancer.

The trends in breast cancer incidence in young women previously presented by Tarone in Table 1 of his article (Tarone 2006) do not refute a possible effect of DDT exposure in childhood. Successive birth cohorts of women diagnosed at 20–39 years of age between 1975 and 2002 (Table 1; Tarone 2006) experienced decreasing DDT exposure in childhood (birth years 1941–1982) because DDT use began in 1945, peaked in 1959, and was banned in 1972 in the United States (U.S. Environmental Protection Agency 1975). Successive birth cohorts of women diagnosed at 40–49 years of age between 1990 and 2002 (Table 1 in Tarone 2006) were all exposed to DDT in childhood (birth years 1941–1962); therefore, breast cancer trends for these birth cohorts are not informative for investigating effects of DDT exposure in childhood. Further, we agree with Weiss (2007) that trends in invasive disease and mortality cannot be interpreted without consideration of the rising incidence of \textit{in situ} disease and its successful treatment, which would reduce incidence of invasive disease and mortality.

The authors declare they have no competing financial interests.

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Romieu I, Hernandez-Avila M, Lazcano-Ponce E, Weber JP, Dzewailly E. 2000. Breast cancer, lactation history and the risks of these ingredients to human health for their use in cosmetic products. This was noted by Salvido (2005) in his response to the work of Luckenbach and Epel (2005). These ingredients have also been evaluated by the European Chemicals Bureau (ECB) for determination of their environmental hazards [persistence, bioaccumulation, and toxicity (PBT)], and the ECB has determined that these materials are not PBTs (European Chemical Bureau 2004). In addition, the SCCNFP issued favorable opinions on both AHTN and HHCkB, finding them safe for use in cosmetic products (SCCNPFP 2002a, 2002b). Further, there have been >40 publications in peer-reviewed scientific journals pertaining to the human health and environmental safety of polycyclic musks (references available upon request).

As a peer-reviewed journal whose stated mission is to present the best science in an objective manner, we are disappointed with your continued lack of objectivity and inability to collect the necessary information to present a true perspective of the science. It would appear that your news staff needs to perform more thorough research in preparing their reports and that your peer-review process may be incomplete.

The RIFM, a nonprofit organization whose research is governed by an independent
expert panel, was established to provide the research and testing necessary to assure the safety of ingredients used in the creation of fragrances. The RIFM has been in existence for > 40 years and has well-established relationships with academia; it is also well known among many regulatory agencies around the world for publishing its work in the peer-reviewed literature. Our organization, as well as others from our industry, are listed on the U.S. Environmental Protection Agency’s website under related links (U.S. EPA 2007).

The authors are employed by the Research Institute for Fragrance Materials, which publishes its work in the peer-reviewed literature under the guidance of an independent scientific panel and receives support from the private sector.

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Editor’s note: Forum articles are short and cannot be all-inclusive of a topic. “The Sweet Scent on Baby’s Breath?” [Environ Health Perspect 115:A491 (2007)] focused on the presence of polycyclic musks in breast milk in the United States, which had never been measured before the study by Kannan and colleagues [Environ Sci Technol 41(11):3815–3820 (2007)]. That said, Smith and Salvito are correct that comment from an industry source should have been included in this article.

Both researchers interviewed for this article [Environ Health Perspect 115:A491 (2007)] expressed concern about the impact of long-term bioaccumulation of polycyclic musks, as well as the lack of full understanding thereof. This concern is shared by others in the field; for example, the most recent Lake Michigan Lakewide Management Plan (http://www.epa.gov/lakemich/2006/index.html) includes six polycyclic musks—including AHTN and HHCB—on a watch list of pollutants to be reviewed in 2008. As researchers look at different end points (such as efflux transporters) or sentinel species (such as mussels), new data will continue to emerge that warrant further investigation, as well as reporting.

The Interaction of Agricultural Pesticides and Marginal Iodine Nutrition Status as a Cause of Autism Spectrum Disorders
doi:10.1289/ehp.11010

Roberts et al. (2007) recently reported on the results of their investigation into the relationship between agricultural pesticides and autism spectrum disorders (ASD) and found an association between organochlorines and ASD. One possible mechanism for this relationship is through thyroid disruption (Cheek et al. 1999). There is evidence to suggest that iodine deficiency might be associated with some of the increase in the reported prevalence/incidence of autism (Sullivan and Mabery 2004). For pregnant women who have a marginal iodine nutrition status, the disruption of the thyroid due to exposure to organochlorines could induce iodine deficiency and result in negative effects on the brain of the developing fetus. The U.S. iodine nutrition status has declined markedly over the last three decades, with the current iodine nutrition status among pregnant women being marginal (Caldwell et al. 2005; Hollowell et al. 1998). Because of the current iodine status of pregnant women, the Public Health Committee of the American Thyroid Association (2006) has recently recommended that all pregnant and lactating women take daily iodine supplements. It is interesting that the ASD case mothers tended to be older and more likely to be non-Hispanic white and non-Hispanic black than controls, which is consistent with a poorer iodine nutrition status in older women and in non-Hispanics in the United States (Caldwell et al. 2005; Hollowell et al. 1998).

Ensuring adequate iodine nutrition status of women, especially throughout pregnancy, is an extremely important public health goal. Given the negative effects of a number of environmental chemicals on the thyroid (Zoeller and Crofton 2000), it becomes increasingly important to ensure that all women have an adequate iodine intake and that the recommended approach to assuring adequate iodine nutrition is through a comprehensive iodized salt program (International Council for Control of Iodine Deficiency Disorders/United Nations Children’s Fund /World Health Organization 2001; Sullivan 2007).

The author is a board member of the International Council for the Control of Iodine Deficiency Disorders.

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Editor’s note: In accordance with journal policy, Roberts et al. were asked whether they wanted to respond to this letter, but they chose not to do so.