voedsel en waren autoriteit

Ministerie van Landbouw, Natuur en Voedselkwaliteit
T.a.v. mw. G. Verburg
Kamer 9H04
POSTBUS 20401
2500 EK 'S-GRAVENHAGE

onderwerp
Nanotechnologie

datum
30 januari 2008
ons kenmerk
VWA/2008/3334

pagina
1 \ 2

behandeld door

Geachte Minister,

Nanotechnologie is een technologie in opkomst. Niet alleen het bedrijfsleven, maar ook de overheid investeert veel in de ontwikkeling van nanotechnologie. Er komen steeds meer consumentenproducten op de markt die gebruik maken van nanodeeltjes. Kunststoffen worden bijvoorbeeld sterker en textiel wordt beter water- en vuilafstotend. Daarnaast ziet de voedingsmiddelenindustrie steeds meer toepassingsmogelijkheden.

Om mogelijke risico van deze ontwikkeling in beeld te brengen, heeft Bureau Risicobeoordeling van VWA onderzoek laten uitvoeren op twee terreinen: nanodeeltjes in consumentenproducten en in voedsel. Over beide onderwerpen heeft de directeur van Bureau Risicobeoordeling adviezen uitgebracht, die ik u hierbij aanbied. Ik onderschrijf beide adviezen.

Bureau Risicobeoordeling zal, naast het leveren van een inbreng in de Nederlandse onderzoeksagenda, onderzoek op het gebied van de veiligheid van toepassingen van nanodeeltjes kritisch blijven volgen. Voor zover het budget dit toelaat zal VWA ook eigen risico-onderzoek uitzetten bij kennisinstituten. Daarnaast zal Bureau Risicobeoordeling Nederlandse activiteiten afstemmen met zusterautoriteiten en met EFSA, zodat doublures worden voorkomen en synergie in onderzoek, kennis en expertise kan worden bewerkstelligd. Naarmate meer kennis beschikbaar komt om mogelijke risico's van nanodeeltjes in voedsel en consumentenproducten te kunnen beoordelen, zal Bureau Risicobeoordeling specifieke adviezen aan u uitbrengen.

Voedsel en Waren Autoriteit
Directie Bedrijfsvoering
Prinses Beatrixlaan 2
2595 AL Den Haag
Postbus 19506
2500 CM Den Haag
Telefoon (070) 448 48 48
Fax (070) 448 47 47
www.vwa.nl

Hoogachtend,
Voedsel en Waren Autoriteit

drs. A.M.W. Kleinmeulman
Inspecteur-Generaal

Bijlage(n):
4

Kopie aan:
- De Minister VWS. dr. A. Klink
- DG VWS,dhr. ir. J.I.M. de Goeij
- DG LNV. mw. ir. A.M. Burger
- Directeur VD/LNV. mw. mr. A. Oppers
- Directeur VGP/VWS mw. drs. A.M. van Bolhuis
- Directeur Toezichtsbeleid en Communicatie VWA, dhr. dr. J.J.Ende
- VWS, mw. Van der Kamp
- VWS, mw. Van Mulukom

voedsel en waren autoriteit

Ministerie van Volksgezondheid, Welzijn en Sport
T.a.v. dhr dr. A. Klink
POSTBUS 20350
2500 EJ 'S-GRAVENHAGE

onderwerp
Nanotechnologie

datum
30 januari 2008
ons kenmerk
VWA/2008/3334

behandeld door

Geachte Minister,

Nanotechnologie is een technologie in opkomst. Niet alleen het bedrijfsleven, maar ook de overheid investeert veel in de ontwikkeling van nanotechnologie. Er komen steeds meer consumentenproducten op de markt die gebruik maken van nanodeeltjes. Kunststoffen worden bijvoorbeeld sterker en textiel wordt beter water- en vuilafstotend. Daarnaast ziet de voedingsmiddelenindustrie steeds meer toepassingsmogelijkheden.

Om mogelijke risico van deze ontwikkeling in beeld te brengen, heeft Bureau Risicobeoordeling van VWA onderzoek laten uitvoeren op twee terreinen: nanodeeltjes in consumentenproducten en in voedsel. Over beide onderwerpen heeft de directeur van Bureau Risicobeoordeling adviezen uitgebracht, die ik u hierbij aanbied. Ik onderschrijf beide adviezen.

Bureau Risicobeoordeling zal, naast het leveren van een inbreng in de Nederlandse onderzoeksagenda, onderzoek op het gebied van de veiligheid van toepassingen van nanodeeltjes kritisch blijven volgen. Voor zover het budget dit toelaat zal VWA ook eigen risico-onderzoek uitzetten bij kennisinstituten. Daarnaast zal Bureau Risicobeoordeling Nederlandse activiteiten afstemmen met zusterautoriteiten en met EFSA, zodat doublures worden voorkomen en synergie in onderzoek, kennis en expertise kan worden bewerkstelligd. Naarmate meer kennis beschikbaar komt om mogelijke risico's van nanodeeltjes in voedsel en consumentenproducten te kunnen beoordelen, zal Bureau Risicobeoordeling specifieke adviezen aan u uitbrengen.

Voedsel en Waren Autoriteit
Directie Bedrijfsvoering
Prinses Beatrixlaan 2
2595 AL Den Haag
Postbus 19506
2500 CM Den Haag
Telefoon (070) 448 48 48
Fax (070) 448 47 47
www.vwa.nl

Hoogachtend,
Voedsel en Waren Autoriteit

drs. A.M.W. Kleinmeulman
Inspecteur-Generaal

Bijlage(n):
- 4

Kopie aan:
- De Minister LNV, mw. G. Verburg
- DG VWS,dhr. ir. J.I.M. de Goeij
- DG LNV. mw. ir. A.M. Burger
- Directeur VD/LNV. mw. mr. A. Oppers
- Directeur VGP/VWS mw. drs. A.M. van Bolhuis
- Directeur Toezichtsbeleid en Communicatie VWA, dhr. dr. J.J.Ende
- VWS, mw. Van der Kamp
- VWS, mw. Van Mulukom

voedsel en waren autoriteit
bureau risicobeoordeling

Advies van de directeur Bureau Risicobeoordeling Aan de minister van LNV en de minister van VWS

onderwerp
Nanodeeltjes in voedsel

datum
29 januari 2008
ons kenmerk
VWA/BuR/2007/54281

pagina
1 \ 12

behandeld door

Samenvatting

Toepassingen van nanotechnologie in voedings- en verpakkingsmiddelen lijken een grote vlucht te gaan nemen. Voordat nanodeeltjes op grote schaal worden toegepast is het belangrijk om inzicht te krijgen in mogelijke nadelige gezondheidseffecten van deze toepassingen. Kennis ontbreekt over wat de gevolgen zijn van nano-afmetingen voor de toxicokinetiek en toxicodynamiek van stoffen. Met name voor de bewust geproduceerde deeltjes die niet oplosbaar of afbreekbaar zijn, is er reden tot bezorgdheid. Om de veiligheid van nanodeeltjes te kunnen beoordelen, moet kennis worden gegenereerd over fysisch-chemische eigenschappen, zoals deeltjesgrootte, vorm, oppervlakte-eigenschappen en dergelijke die van invloed kunnen zijn op de toxiciteit. Bovendien moeten hiertoe, en voor wetgeving, standaard dosismeeteenheden worden gedefinieerd.

Bureau Risicobeoordeling adviseert:

1. *onderzoek te stimuleren naar de veiligheid van voedsel dat nanodeeltjes bevat, waar mogelijk in internationale afstemming. Dit onderzoek vraagt om een investering in:*
   a. *meetmethoden en meetapparatuur;*
   b. *onderzoek naar kennislacunes: blootstelling, opname, verdeling en effecten van nanodeeltjes;*
2. *voedingsmiddelen of voedselingrediënten die (bewust geproduceerde) nanodeeltjes bevatten als 'nieuw' te beschouwen zodat de Verordening inzake nieuwe voedingsmiddelen en nieuwe voedselingrediënten (EC/258/97) van toepassing is;*
3. *de veiligheid van additieven en aroma's die nanodeeltjes bevatten te beoordelen ook als het gaat om nanoformuleringen van eerder toegelaten producten.*

Inleiding

Nanotechnologie kan worden beschreven als het geheel van kennis, vaardigheden en apparatuur dat nodig is om op een schaal van 1 tot 100 nm functionaliteit te creëren waarbij gebruik wordt gemaakt van de specifieke eigenschappen van materie op nanoschaal (Kampers 2004). Nanotechnologie maakt het mogelijk om moleculen en atomen te manipuleren (de diameter van een enkel atoom is ongeveer 0,1 nm) en de deeltjesgrootte te verkleinen waardoor nieuwe toepassingen en structuren op velerlei

Voedsel en Waren Autoriteit
Bureau Risicobeoordeling
Prinses Beatrixlaan 2
2595 AL Den Haag
Postbus 19506
2500 CM Den Haag
Telefoon (070) 448 40 72
Fax (070) 448 40 71
www.vwa.nl

terreinen mogelijk worden. Europa behoort qua onderzoeksinvestering tot de top drie van de wereld, na Japan en de Verenigde Staten (Joseph en Morrison 2006). Ook in Nederland wordt vanuit de ICES/KIS-middelen veelal fundamenteel onderzoek gefinancierd, met name in de vorm van de programma's MicroNed en NanoNed. Naast aandacht voor mogelijkheden en toepassingen, hoort er ook aandacht te zijn voor de veiligheid van nanotechnologie (U.S. Food and Drug Administration 2007).

De VWA is verantwoordelijk voor het (laten) beoordelen van de veiligheid van voedingsmiddelen, en ook voor het toezicht hierop. Daartoe moet VWA kunnen nagaan in hoeverre producenten hun verantwoordelijkheid invullen om veilige voedingsmiddelen op de markt te brengen.
Dit advies is gericht op de veiligheid van nanodeeltjes in voedingsmiddelen en op het beleid dat de overheid hiervoor zou kunnen ontwikkelen. Hierbij komen achtereenvolgens de volgende vragen aan bod:

- Van welke voedingsmiddelen is blootstelling aan nanodeeltjes te verwachten voor de consument?
- Wat zijn de mogelijke gevaren van nanodeeltjes in voedsel?
- Hoe kan de veiligheid van nanoproducten worden onderzocht? Welke kennislacunes bestaan er?
- Welke wetgeving is van toepassing op voedsel dat nanodeeltjes bevat of met hulp van nanotechnologie is geproduceerd?

Werkwijze
Bureau Risicobeoordeling van VWA heeft de ontwikkeling van nanotechnologie gesignaleerd en besloten pro-actief te reageren. Eind 2006 heeft het Bureau daarom een deskundigenplatform ingesteld van onderzoekers en beleidsambtenaren die zich met veiligheidsaspecten van nanotechnologie bezighouden. Ook heeft het Bureau in samenwerking met het Rathenau Instituut in juni 2007 een workshop georganiseerd voor wetenschappers, beleidsmakers en vertegenwoordigers van maatschappelijke organisaties over maatschappelijke vragen rond nanovoedselveiligheid.

Als basis voor het voorliggende advies is een document van RIKILT/RIVM (Bouwmeester et al. 2007; bijlage 1) gebruikt, is de literatuur doorzocht zijn deskundigen geconsulteerd en congressen en workshops bijgewoond. Een gelijktijdig uitgebracht advies heeft betrekking op nanodeeltjes in consumentenproducten.

Er is nog geen algemeen aanvaarde definitie van nanotechnologie. In dit advies worden de definities uit bijlage 2 (British Standards Institution 2005) gebruikt.

Nanotechnologie in de voedselketen en nanodeeltjes in de voeding
Nanotechnologie kan worden ingezet voor proces- en productinnovaties ter verbetering van de gezondheidswaarde, de veiligheid, de smaak en de aantrekkelijkheid van voedingsmiddelen Belangrijke toepassingen in voedingsmiddelen zijn bijvoorbeeld het

combineren van ingrediënten die anders niet samen kunnen worden verwerkt, of een gerichte en in de tijd gefaseerde afgifte van bioactieve stoffen of smaak- en geurstoffen (Prisma & Partners en MinacNed 2006). Daarnaast kan nanotechnologie bijdragen aan het goedkoper en efficiënter produceren van voedingsmiddelen door minder water, energie en chemicaliën te gebruiken. Voor een overzicht van (mogelijke) toepassingen van nanotechnologie in de voedselketen, zie bijlage 3. Het overzicht is gebaseerd op informatie op of over de producten. Via internet zijn verschillende producten te koop, voornamelijk supplementen, waarvan wordt aangegeven dat ze nanodeeltjes bevatten (zie bijvoorbeeld www.nanoshop.com).

Veel bestanddelen in voedingsmiddelen hebben een nanoschaal. Voorbeelden zijn: caseïne in melk, thee, emulsies, micellen en liposomen. Nanodeeltjes zijn altijd al van nature aanwezig geweest in ons voedsel. Maar nu is er voor het eerst sprake van door de mens gericht geproduceerde ('engineered') nanodeeltjes. Hierbij kan onderscheid worden gemaakt tussen twee typen nanodeeltjes:

- Nanodeeltjes bestaande uit stoffen die van nature ook in aanzienlijke concentraties voorkomen in voedingsmiddelen en die oplosbaar zijn of door het lichaam worden afgebroken. Voorbeelden zijn nano-afgiftesystemen, die gewoonlijk worden opgebouwd uit peptide- of lipidemonomeren (nanocapsules) en worden toegepast voor een gerichte afgifte van bioactieve stoffen (Weiss et al. 2006), en het inbouwen van nanocapsules met visolie in brood. Deze capsules gaan pas open in de maag en voorkomen de onplezierige smaak van visolie. Synthetische nanodeeltjes van lycopeen zijn een voorbeeld van nanodeeltjes die ontwikkeld en getest zijn, en geaccepteerd met een GRAS-status door de FDA voor gebruik in voedingsmiddelen in de Verenigde Staten (Institute of Food Science and Technology Trust Fund 2006).
- Non-food nanodeeltjes. Dit zijn niet afbreekbare of oplosbare deeltjes. Voorbeelden van non-food nanodeeltjes in voedingsmiddelen zijn: zilver, silicaat, siliciumoxide en goud. Een voorbeeld is het toevoegen van siliciumdioxide en polystyreenbolletjes aan voedingsmiddelen om smaak en textuurgewaarwordingen te versterken (Engelen et al. 2005). Ook zouden non-food nanodeeltjes vanuit verpakkingsmiddelen in het voedsel terecht kunnen komen.

Is er gevaar?

Nanodeeltjes kunnen worden opgenomen via het maagdarmkanaal (Hoet et al. 2004), maar er bestaan onzekerheden over absorptie, verdeling, afbraak en uitscheiding. Wel is er aangetoond dat cellen nanodeeltjes kunnen opnemen. Nanodeeltjes kunnen weefsels bereiken die grotere, qua chemische samenstelling vergelijkbare, deeltjes niet kunnen bereiken. Geïnhaleerde nanodeeltjes kunnen in de bloedbaan komen en de bloed-hersenbarrière passeren (Oberdörster et al. 2004). De mogelijkheid bestaat dat de deeltjes reageren met DNA en eiwitten in cellen of oxidatieve stress veroorzaken. Dit zou ontstekingen en cel- en DNA-beschadigingen kunnen veroorzaken (Oberdörster et

al. 2005, Nel et al. 2006) met degeneratieve en andere ziekten als gevolg (Borm et al. 2006, Lomer et al. 2002, Lomer et al. 2004).

Onderzoek naar de veiligheid van nanotechnologie
Bouwmeester et al. (2007) concluderen dat de resultaten van de beschikbare toxiciteitsonderzoeken aangeven dat acute orale blootstelling aan nanodeeltjes kan leiden tot toxische effecten. Echter er is geen informatie over chronische toxiciteit van blootstelling aan lage doseringen. Blootstelling aan nanodeeltjes kan blootstelling aan een hele reeks afmetingen en vormen van deeltjes inhouden. De bestaande aanpak van risicobeoordelingen voor stoffen is daarom niet zondermeer hanteerbaar. Nader onderzoek is nodig om na te gaan of nanodeeltjes vergeleken met de conventionele stoffen, nadelige gezondheidseffecten hebben. Tot nu toe is slechts een klein aantal specifieke nanodeeltjes onderzocht in een beperkt aantal testsystemen (Borm et al. 2006). Nanodeeltjes die oplosbaar of afbreekbaar zijn (dus worden herkend door de lichaamsenzymen) vormen wellicht geen probleem. Er is met name zorg over ongebonden, niet oplosbare of afbreekbare deeltjes. De toxiciteit van deze deeltjes lijkt te worden bepaald niet alleen door chemische structuur, maar ook door bijvoorbeeld deeltjesgrootte, oppervlak, verschijningsvorm en aantal (Maynard 2006). Om na te gaan of er een risicobeoordeling nodig is voor een bepaald product, kan de beslisboom van SCENIHR (2005) worden gebruikt. Gezien de grote aantallen stoffen en de vele vormen en verschillende deeltjesgroottes, zal een formele risicobeoordeling voor bewust geproduceerde nanomaterialen van geval tot geval moeten worden uitgevoerd (Colvin 2003).

Het is mogelijk dat behalve bewust geproduceerde en toegevoegde nanodeeltjes ook ongewenste nanodeeltjes (contaminanten of bijproducten) in voedingsmiddelen terechtkomen. Voorbeelden zijn residuen van bestrijdingsmiddelen en deeltjes die migreren uit verpakkingen.

Communicatie
Uit de workshop over nanovoedselveiligheid, die werd georganiseerd samen met het Rathenau Instituut in juni 2007, bleek dat er grote behoefte bestaat aan een dialoog over nanotechnologie en aan informatie-uitwisseling tussen alle betrokken groeperingen. Een gebrek aan kennis over nanotechnologie en dus een onvoldoende inzicht in de voor- en nadelen van marktintroductie kan tot onzekerheid leiden bij burgers en daardoor tot afwijzing van producten met nanodeeltjes of zelfs van nanotechnologie als geheel.

Wetgeving
De Algemene Levensmiddelen Verordening (EC/178/2002) gaat uit van het voorzorgsbeginsel: als de mogelijkheid van schadelijke gevolgen voor de gezondheid is geconstateerd, maar er nog wetenschappelijke onzekerheid heerst, kunnen voorlopige maatregelen voor risicomanagement worden vastgesteld. Hieraan kan invulling worden

gegeven door producten die nanomateriaal bevatten als 'nieuw' te beschouwen, waardoor de Verordening inzake nieuwe voedingsmiddelen en nieuwe voedselingrediënten (EC/258/97) van toepassing wordt. Onder deze verordening vallen voedingsmiddelen en voedselingrediënten met een nieuwe of doelbewust gemodificeerde primaire molecuulstructuur en voedingsmiddelen en voedselingrediënten waarop een weinig gebruikt productieprocédé is toegepast, voor zover dit procédé wijzigingen in de samenstelling of de structuur van de voedingsmiddelen of voedselingrediënten veroorzaakt die significant zijn voor hun voedingswaarde, hun metabolisme of hun gehalte aan ongewenste stoffen. De Verordening voorziet in aanvullende specifieke voorschriften inzake etikettering. Levensmiddelenadditieven en aroma's zijn van deze verordening uitgesloten; hierop zijn andere Richtlijnen van toepassing, die voorzien in het beoordelen van nieuwe additieven en aroma's. Echter deze Richtlijnen voorzien niet eenduidig in een hernieuwde goedkeuring van bestaande en toegelaten additieven en aroma's die op nanoschaal worden geproduceerd. Het Institute of Food Science and Technology Trust Fund (IFST) stelt voor om nanodeeltjes die worden gebruikt als additieven op het etiket op te nemen via een aanpassing van het E-nummer-systeem, bijvoorbeeld door aan een bestaand E-nummer een subscript 'n' toe te voegen (IFST 2007).

Ook het EU Scientific Committee on Emerging and Newly-Identified Health Risks (SCENIHR) concludeert dat het EU-regelkader in principe ook nanotechnologieën dekt (SCENIHR 2007). IFST in Groot Brittannië beveelt aan om nanodeeltjes te behandelen als nieuwe, potentieel schadelijke materialen totdat hun veiligheid is bewezen (het voorzorgsprincipe). De Amerikaanse Environmental Protection Agency (EPA) staat op het standpunt dat natuurlijke nanoproducten in het algemeen niet in de wetgeving behoeven te worden betrokken. EPA beschouwt bovendien nanodeeltjes die ontstaan als toevallige bijproducten van menselijke activiteit en nanodeeltjes afkomstig van natuurlijke processen niet als producten van nanotechnologie, alhoewel deze deeltjes dezelfde effecten kunnen hebben (Davies 2007).

Risicobeoordeling

Kennis ontbreekt om een echte risicobeoordeling uit te voeren. Voedingsmiddelen met nanodeeltjes zijn mogelijk wezenlijk anders dan vergelijkbare voedingsmiddelen zonder nanodeeltjes. Omdat bestaande limieten of drempelwaarden niet van toepassing hoeven te zijn voor nanodeeltjes, dienen de dossiervereisten te worden uitgebreid met o.a. het toevoegen van informatie over fysisch-chemische parameters, zoals deeltjesgrootte, vorm, oppervlakte-eigenschappen en andere (functionele) eigenschappen die van invloed kunnen zijn op de toxiciteit. Daarnaast is het de vraag of standaard toxicologische testen voldoen. Bouwmeester et al. (2007) denken van wel, maar vragen om extra aandacht voor neurotoxiciteit, reproductietoxiciteit, mutageniteit en allergeniteit. De Gezondheidsraad (2007) adviseert de eisen voor de dossiers op Europees niveau te harmoniseren.

Conclusies

- Afgaand op namen van producten, informatie op etiketten of op websites, blijkt dat er in de voedselketen een groot aantal producten met ('engineered') nanodeeltjes kunnen worden aangetroffen.
- Er is gebrek aan kennis over de gevolgen van nanoafmetingen voor de toxicokinetiek en toxicodynamiek van stoffen.
- Er kan in principe niet worden aangenomen dat voedingsmiddelen of voedselingrediënten met 'engineered' nanodeeltjes veilig zijn. Dit kan alleen worden vastgesteld door een veiligheidsbeoordeling die specifiek op de nanovorm is gericht.
- Voor oplosbare, afbreekbare nanodeeltjes die van nature voorkomen in voedingsmiddelen (emulsies, micellen, liposomen) kan worden aangenomen dat ze veilig zijn.
- Algemeen aanvaarde definities van nanotechnologie en van alle afgeleide begrippen, zoals nanodeeltjes, nanomaterialen, etc. ontbreken.
- Een open dialoog met de samenleving over mogelijkheden en risico's is belangrijk en kan onnodige onzekerheid bij burgers voorkomen.

Advies

Gezien het bovenstaande adviseer ik:

1. *onderzoek te stimuleren naar de veiligheid van voedsel dat nanodeeltjes bevat, waar mogelijk in internationale afstemming. Dit onderzoek vraagt om een investering in:*
   a. *meetmethoden en meetapparatuur;*
   b. *onderzoek naar kennislacunes: blootstelling, opname, verdeling en effecten van nanodeeltjes;*
2. *voedingsmiddelen of voedselingrediënten die (bewust geproduceerde) nanodeeltjes bevatten als 'nieuw' te beschouwen zodat de Verordening inzake nieuwe voedingsmiddelen en nieuwe voedselingrediënten (EC/258/97) van toepassing is;*
3. *de veiligheid van additieven en aroma's die nanodeeltjes bevatten te beoordelen ook als het gaat om nanoformuleringen van eerder toegelaten producten.*

Hoogachtend,

Prof. Dr. E.G. Schouten
Directeur Bureau Risicobeoordeling

Bijlagen:
1. Rapport van RIKILT/RIVM
2. Definities
3. Toepassingen van nanotechnologie in de voedselketen
4. Kennisvragen

Referenties

Borm PJA, Robbins D, Haubold S, et al. The potential risks of nanomaterials: a review carried out for ECETOC. Particle Fibre Toxicol 2006;3:11, 35 p.

Bouwmeester H, Dekkers S, Noordam M, Hagens W, Bulder A, de Heer C, ten Voorde S, Wijnhoven S, Sips A. Health impact of nanotechnologies in food production. Wageningen: RIKILT/RIVM, September 2007.

British Standards Institution (BSI). Vocabulary – Nanoparticles. London: BSI, 25 May 2005; ICS 01.040.07;07.030; PAS 71:2005.

Colvin VL. The potential environmental impact of engineered nanomaterials. Nature Biotechnol 2003;21:1166-1170.

Davies JC. EPA and nanotechnology: oversight for the 21st century. Woodrow Wilson International Center for Scholars, Project on Emerging Nanotechnologies (PEN 9), May 2007.

Engelen L, de Wijk RA, van der Bilt A, Prinz JF, Janssen AM, Bosman F. Relating particles and texture perception. Physiol Behavior 2005;86:111-117.

Gezondheidsraad. Veiligheidsbeoordeling van nieuwe voedingsmiddelen (2). Den Haag: Gezondheidsraad, 2007; publicatienr 2007/23.

Hoet PHM, Brüske-Hohlfeld I, Salata OV. Nanoparticles – known and unknown health risks. J Nanobiotechnol 2004;2:12, 15 p.

Institute of Food Science & Technology Trust Fund (IFST). Information Statement Nanotechnology. February 2006.

Joseph T, Morrison M. Nanotechnology in agriculture and food (a Nanoforum Report). Nanoforum.org. European Nanotechnology Gateway, May 2006.

Kampers FWH. Potentiele risico's van bio-nanotechnologie voor mens en milieu. Redactie: Sudhölter EJR. Oriëntatierapport in opdracht van COGEM. Wageningen, mei 2004.

Lomer MC, Thompson RP, Powell JJ. Fine and ultrafine particles of the diet: influence on the mucosal immune response and association with Crohn's disease. Proc Nutr Soc 2002;61:123-130.

Lomer MCE. Hutchinson C, Volkert S, Greenfield SM, Catterall A, Thompson RPH, Powell JJ. Dietary sources of inorganic microparticles and their intake in healthy subjects and patients with Crohn's disease. Br J Nutr 2004;92:947-955.

Maynard AD. Nanotechnology: A research strategy for addressing risk. Woodrow Wilson International Center for Scholars. Project on Emerging Nanotechnologies (PEN 3), July 2006.

Nel A, Xia T, Mädler L, Li N. Toxic potential of materials at the nanolevel. Science 2006;311;622-627.

Oberdörster G, Maynard A, Donaldson K, et al. Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy. Particle Fibre Toxicol 2005;2:8, 35 p.

Oberdörster G, Sharp Z, Atudorei V, et al. Translocation of inhaled ultrafine particles to the brain. Inhal Toxicol 2004;16:437-445.

Prisma & Partners, MinacNed. Roadmap Microsystem- & Nanotechnology in Food & Nutrition. Juli 2006.

Scientific Committee on Emerging and Newly-Identified Health Risks (SCENIHR). Opinion on the appropriateness of existing methodologies to assess the potential risks associated with engineered and adventitious products of nanotechnologies. European Commission, SCENIHR, 28-29 September 2005.

Scientific Committee on Emerging and Newly-Identified Health Risks (SCENIHR). Opinion on the appropriateness of the risk assessment methodology in accordance with the technical guidance documents for new and existing substances for assessing the risks of nanomaterials. European Commission, June 2007.

U.S. Food and Drug Administration (FDA). Nanotechnology Task Force Report. Nanotechnology. July 25, 2007.

Weiss J, Takhistov P, McClements DJ. Functional materials in food nanotechnology. J Food Sci 2006;71:R107-R116.

Bijlagen

Bijlage 1 - Rapport RIKILT/RIVM: Bouwmeester H, Dekkers S, Noordam M, Hagens W, Bulder A, de Heer C, ten Voorde S, Wijnhoven S, Sips A. Health impact of nanotechnologies in food production. Wageningen: RIKILT/RIVM, October 2007.

Bijlage 2 - Definities

De definities die Department of Trade and Industry (DTI) in samenwerking met British Standards Institution (BSI) hanteert, en die in dit advies worden gebruikt, zijn als volgt (BSI 2005):

- Nanomaterial: material with one or more external dimensions, or an internal structure, on the nanoscale, which could exhibit novel characteristics compared to the same material without nanoscale features.
- Nanoparticle: particle with one or more dimensions at the nanoscale.
- Nanoscale: having one or more dimensions of the order of 100 nm or less.
- Nanoscience: study of phenomena and manipulation of materials at atomic, molecular en macromolecular scales, where properties differ significantly from those at a larger scale.
- Nanotechnology: design, characterization, production and application of structures, devices and systems by controlling shape and size at the nanoscale.
- Engineered nanoparticles: nanoparticles manufactured to have specific properties or a specific composition.

Bijlage 3 - Toepassingen van nanotechnologie in de voedselketen

Ketenfase	Toepassing	Nanotechnologie/type nanodeeltje	Functie
Landbouw-productie	Nanosensoren	Nanospray op voedingsmiddelen	Binden en kleuren van micro-organismen
		(Kleine) apparaten	Detecteren van contaminanten, etc.
		Ingebouwd in verpakkingsmateriaal	Detecteren van achteruitgang kwaliteit
	Pesticiden (delivery systems)	Nano-emulsies en -capsules	Grotere doelmatigheid, wateroplosbaarheid en hechting aan gewas
		Gerichte afgifte van capsulaten	Gerichte (lokale) afgifte
	Waterzuivering/ grondontsmetting	Filters met nanoporiën	Verwijderen van pathogene en contaminanten
		Nanodeeltjes	Verwijderen of katalyseren van contaminantoxidatie
Productie en verwerking	Voedselproductie	Nanokeramische hulpmiddelen	Groot reactief oppervlak
	Koelkasten, opslagcontainers, voedselbereidings-materiaal	Ingebouwde nanodeeltjes, voornamelijk zilver, soms zinkoxide	Antibacteriële coating van opslag- en voedsel behandelingsinrichtingen
Conservering en verpakking	Voedselproducten	Nano-zilver sprays	Antibacterieel
		Ingebouwde sensoren	Detecteren van kwaliteitsverlies, monitoren van opslag
		Ingebouwde nanodeeltjes	Toename van barrière-eigenschappen, sterkte materialen
		Ingebouwde actieve nanodeeltjes	Wegvangen van zuurstof, voorkomen van pathogenengroei
Functionele voedingsmid-delen, consumptie	Supplementen, additieven	Colloïdale metalen nanodeeltjes	Verhogen van opname
		Delivery systems 'nanoclusters'	Beschermen en gerichte afgifte van inhoud
		Nanoafmeting voedsel/dranken (voedingsstoffen)	Verhoogde opname
	Voedingsmiddelen	Inerte deeltjes, dragers, andere toepassingen	

Bron: Bouwmeester et al. 2007

Bijlage 4 - Kennisvragen

Een onderzoeksprogramma om de huidige kennislacunes die een risicobeoordeling en eenduidige wetgeving belemmeren, te dichten moet inzicht verschaffen in de volgende onderwerpen (Bouwmeester et al. 2007):

- Het karakteriseren en meten van nanodeeltjes zodat een realistische schatting van de blootstelling kan worden verkregen en dosis-responsrelaties kunnen worden vastgesteld.
- Het bepalen van meeteenheden omdat de dosis moet kunnen worden gekwantificeerd voor gevarenkarakterisatie en blootstellingsbepalingen en om de interpretatie van onderzoeken en van wettelijke kaders mogelijk te maken.
- Het onderzoeken van de effecten van nanodeeltjes omdat die anders kunnen zijn dan van conventionele stoffen. Als er bewijs is voor de opname van nanodeeltjes, moet de verdeling worden bestudeerd (met speciale aandacht voor die delen van het menselijk lichaam die worden beschermd door barrières, zoals de bloed-hersenbarrière en de placenta), het metabolisme en de uitscheiding.
- Het onderzoeken van de blootstelling van de consument aan nanodeeltjes. Nagegaan moet worden welke producten die nanodeeltjes bevatten op de markt zijn en welk type deeltjes wordt gebruikt in deze producten en waar effecten op de biobeschikbaarheid optreden. Ook de mogelijke migratie van nanodeeltjes uit materialen in contact met voedsel dient te worden onderzocht.
- Het inventariseren van wetenschappelijke behoeften en datavereisten voor pre- en postmarketing situaties.
- Het toetsen van de adequaatheid van de richtlijnen voor toxicologisch onderzoek en methoden voor risicobepaling met speciale aandacht voor neurotoxiciteit, reproductietoxiciteit en embryotoxiciteit, mutageniteit en allergie.

Er moet ook verder worden geïnvesteerd in nieuw, exploratief onderzoek, ook al om de vragen die we nu nog niet kunnen voorzien, in de toekomst te kunnen beantwoorden.

roject number: 772.308.01
Project title: Health risks of application of nanotechnologies and nanoparticles within the food production chain.

Project leader: H. Bouwmeester

Report 2007.014 September 2007

Health impact of nanotechnologies in food production

This report is a co-production of RIKILT and RIVM

H. Bouwmeester[1], S. Dekkers[2], M. Noordam[1], W. Hagens[2], A. Bulder[1], C. de Heer[2], S. ten Voorde[1], S. Wijnhoven[2], A. Sips[2]

[1] RIKILT – Institute of Food Safety, Wageningen UR
[2] National Institute of Public Health & the Environment; Center for Substances and Integrated Risk Assessment

Advisory team:
I. M.C.M. Rietjens (WUR-toxicology), W. H. de Jong (RIVM-Laboratory for Health Protection Research), A. A.C.M. Peijnenburg, H.J.P. Marvin (WUR-RIKILT)

RIKILT – Institute of Food Safety
Wageningen University and Research Centre
Bornsesteeg 45, 6708 PD Wageningen, NL
P.O. Box 230, 6700 AE Wageningen, NL
Tel: +31 317 475422
Fax: +31 317 417717
Internet: www.rikilt.wur.nl

RIVM – National Institute for
Public Health & the Environment
A. v. Leeuwenhoeklaan 9, NL
P.O. Box 1, 3720 BA Bilthoven, NL
Tel: + 31 30 274 9111
Fax: +31 30 274 4450
www.rivm.nl

The research described in this report was commissioned and funded by the Dutch and Consumer Products Safety Authority, Office for Risk Assessment

Distribution list:

Food and Consumer Product Safety Authority (VWA) (dr. D. van Aken, dr.ir. J. Castenmiller, dr. H. Noteborn, prof.dr. E. Schouten, drs. J. Cornelese, dr. W. van Eck, dr. W. van der Sande, dr.ir. P. Bragt, dr. F. van Duijne, drs. F. Gaikema)
Dutch Ministry of Agriculture, Nature and Food Quality (minister G. Verburg, ir. B. van den Assum, dr.ir. R. Donkers, mr. A. Oppers, dr.ir. M. Kool, dr. J.A. Hoekstra)
Ministry of Housing, Spatial Planning and the Environment (VROM) (dr. T. van Teunenbroek, drs. M. Verschuuren)
Ministry of Health, Welfare and Sport (minister A. Kink, ir. J.M.E. van der Kamp, drs. H.F. Storms, drs. D. van Mulukom, drs. J. van de Wijngaard, drs. J. Puiman)
Ministry of Social Affairs and Employment (drs. E. van de Acker)
Ministry of Economic Affairs (drs. F. von Meijenfeldt, ir. A.P. Couzy)
Rathenau Institute (dr. R. van Est, drs. B. Walhout)
Senternovem (dr. R. Visman)
Universität Dusseldorf (prof.dr. P. Borm)
University of Twente (prof.dr. A. Rip)
TNO Quality of Live (dr.ir. M. Rennes, dr. F. Simonis)
Tilburg Institute for Law, Technology, and Society (TILT) (prof.dr. E.J. Koops)
University of Maastricht (prof.dr. W.E. Bijker)
The Health Council of the Netherlands (dr. H.F.G. van Dijk)
Bundesinstitüt für Risikenbewertung (prof.dr. A. Lampe)
European Food Safety Authority (EFSA) (dr. S. Barlow)
European Commission (dr. C. Pattermann, dr. A. Aguilar-Romanillos, dr. D. de Martinis, dr. M. Pilar Aguar Fernández)
Central Science Laboratory (dr. Q. Chaudhry)
Institute of Occupational Medicine (dr. L. Tran, dr. R. Aitken)
Institute of Nanotechnology (dr. M. Morrison, dr. R. Moore)
Institute for Work and Health (dr. M. Riediker)
EMPA (dr. Matthias Roesslein)
Institute of Biochemistry (prof.dr. V. Popov)
VTT Technical Research Centre of Finland (Dr. M. Smolander)

Berufsgenosssenschaftliches Institut für Arbeitsschutz (HVBG-BGIA) (dr. M. Berges)
School of Chemistry & Chemical Biology (UCD) (dr. I. Lynch)
National Institute for Public Health and the Environment (RIVM) (dr. W.H. de Jong, dr.ir. A. Henken, dr.ir. R. Woittiez, dr. M. van Raaij, dr. R. van Leeuwen, dr.ir. M. van Zijverden)
Wageningen University and Research Center (prof.dr.I. Rietjens, dr. G. Alink, prof.dr.mr. B.M.J. van der Meulen, dr. F. Kampers, dr. N. van den Brink, dr. K. Maassen, dr. H. Zuilhof, prof.dr. L. Frewer, dr. E. van der Linden, prof.dr. W. Norde, dr. M. Jongsma, dr. R. van Gorcom)

Preface

Nanotechnology is an emerging technology that will have great impact on product innovation in the coming years. Currently the technology is already used in innovative cosmetic and medical products. In the food industry there is a clear potential for product and process innovation using nanotechnology and nanoparticles. This is exemplified already now by the availability of food products developed by making use of nanotechnology.

It is however the societal responsibility of industry, governments and researchers to get inside in potential risks of the application of this evolving technology. The smaller the particles are the closer they come to the size/structure of natural barriers in nature and our body. Since we currently do not know what this means for the natural barrier functions we can not simply extrapolate our knowledge on the safety of micro- and macro structures and delivery systems to their nano-sized equivalents.

Consumer acceptance of new products or products produced with new technologies has had serious dents in recent years at the introduction of food irradiation technology and genetic modification technology. Consequently both risk evaluation and consumer perception are important issues to be addressed in parallel with the development and application of new technologies. Disregarding these aspects could have dramatic negative aspect not only on the introduction of nanotechnology but also more in general to public perception of new technologies and product innovation.

As a start in this process the Dutch Food and Consumer Product Safety Authority has asked RIKILT-Institute of Food Safety, Wageningen UR and the National Institute for Public Health and the Environment to perform an inventory study on the current use of nanotechnology in food products and give advise on the most relevant safety evaluation issues. This report describes the results of this study. The report is set up in two parts. First you will find an aggregation of the results in the answer to 10 questions. In this part you will also find our suggestions for prioritizing the research that is needed. The second document is a scientific background document.

We hope that this report will be a stimulus for the various stakeholders in the process of a responsible development of this technology in facilitating the necessary research and risk evaluation.

Robert van Gorcom
Dept. Director RIKILT - Institute of Food Safety

André Henken
Director Division Food, Medicines and Consumer Safety - RIVM

Abstract

In the food production chain nanotechnologies will impact food security, packaging materials, delivery systems, bioavailability, and new materials for pathogen detection, thereby contributing to the targets set for achieving the UN Millennium Development Goals. Already yet, food products containing nanoparticles are penetrating the market, with a prominent role for sales via the Internet. This implies that regulatory frameworks and risk assessment should meet criteria for both pre- and post-marketing situations.

As with most new and evolving technologies, potential benefits of nanotechnologies for agriculture, food industry and consumers are emphasized. However, little is known on safety aspects of the application of nanotechnologies in food production and the incorporation of nanoparticles in food products. Therefore, there is a need for swift actions by policy makers and scientists as regulatory frameworks seem to need adaptation and scientists should give input for these adaptations. Their joint actions should facilitate the process of minimizing the health and environmental risks, while stimulating the economic developments of nanotechnologies in the food production chain.

This report gives an overview and an advice for priority of scientific issues that need to be addressed in order to improve the process of risk assessment for nanoparticles in food and in order to gain insight in dossier requirements for nanoparticles in food. The following research topics are considered to contribute pivotally to risk assessment of nanotechnologies and nanoparticles in general and thus also for applications in food products.

- *Characterization of nanoparticles.* The particles have novel properties compared to conventional[1] chemicals. It is important to characterize these properties to enable realistic estimations of consumer exposure. But equally important, this information is needed to establish dose-response relations in toxicology studies. Thus, analytical tools need to be developed for the isolation and characterization of nanoparticles in food and biological matrices.
- *Dose metrics.* This is a very basic issue which affects both interpretation of scientific studies as well as regulatory frameworks. It has become clear that doses of nanoparticles and thus also limit values for nanoparticles cannot be expressed in weight or volume measures as is the case for conventional chemicals. Questions arise whether nanosized particles of their conventional counterparts need their own limit values.
- *Effects of nanoparticles.* The kinetics of nanoparticles may be different compared to conventional chemicals. When there is evidence for uptake, distribution of nanoparticles should be studied more extensively when compared to their conventional counterparts. Of special importance are those parts of the body that are normally protected by barriers like the blood-brain-barrier and placenta.
- *Definition of nanoparticles.* This is not only a formal issue for regulators but also very important for discussion on prioritization of research and exchange of study results between scientists, producers and regulators.
- *Consumer exposure to nanoparticles.* It needs to be studied which products containing nanoparticles are on the market and which type of particles are used, and are being developed.

Specifically for applications of nanoparticles and nanotechnologies in food products are the following issues thought to be relevant:

- *Oral bioavailability*
- *Measurement of nanoparticles in food matrices*

[1] Convential meaning not nano-sized

The proposed research issues should contribute to the development of safe nanotechnology and thus stimulating the economic developments of nanotechnologies. Products containing or generated by means of nanotechnology are already available on the market. It is evident that safety is in the first place the producers' responsibility, however involvement of all relevant stakeholders will be required to protect consumers adequately.

Content

Part A.

Health impact of nanotechnologies in food production:

Food safety issues of nanotechnologies in 10 questions

Introduction

Nanotechnologies have the potential to contribute to the targets set for achieving the UN Millenium Development Goals, particularly in the areas of affordable energy, clean water, human health, and the environment. To bring these promises to fruition, public research programmes have an important role to play in providing greater incentives and encouragement for nanotechnologies that support sustainable development [UN Geo Year Book 2007].

Nanotechnology has also the potential to impact many aspects of food and agricultural systems. Food security, packaging materials, disease treatment, delivery systems, bioavailability, new tools for molecular and cellular biology and new materials for pathogen detection are examples of the important items that are linked with nanotechnology within the food production chain (Chen et al. 2006a; Weiss et al. 2006). Food products containing nanotechnologies are penetrating the market, albeit currently predominantly outside the EU (e.g. Japan, China and the USA). It is however widely anticipated that they will appear on the EU market in the next few years. Currently many products containing nanotechnologies are of course globally available due to sales via the Internet.

As with most new and evolving technologies, much emphasis is on the potential benefits of nanotechnology for agriculture, the food industry and likely the consumer. However, not too much is known on safety aspects of the application of nanotechnologies in food production and the incorporation of nanoparticles (NPs) in food products (Maynard 2006). The rapid emerging of nanotechnology creates therefore a need for swift action by policy makers. Their actions should facilitate the process of minimizing the health and environmental risks. As nanofood products are already on the market and uncertainty about potential risks is large, the need for science-based adaptation of the regulatory frameworks is high.

The aim of this report is to identify knowledge gaps in the expertise needed to make reliable safety or risk assessments for consumer health in case of application of nanotechnology in food production.

To this end first a inventory of products containing nanotechnologies that are currently on the market has been made. In addition an overview of the current knowledge on the potential hazards of NPs has been made based on a review of literature. This resulted in a background report, including detailed discussions on specific topics. Discussion with experts in toxicology, and on the general experimental requirements for dossiers to be submitted for risk and safety assessment of chemicals resulted in the development of a synthesis document.

Outline

The synthesis document is the first part of this report. On the basis of 10 questions covering the most important food safety issues of nanotechnology, knowledge gaps are identified, research issues named and potential impact of research outcomes on quality of risk assessment and regulatory framework identified. Subsequently, this information is applied for formulating a proposal for prioritizing research issues. Within this document reference is made to the second part of this report: the background document. There a scientific background is provided to the identified knowledge gaps. The background document provides an overview of the current-state-of-the-knowledge, without prioritization.

1 In which parts of the food chain are nanotechnologies applied?

Nanotechnology tools are used in the entire food production chain e.g. during cultivation (e.g. pesticides), industrial processing or packaging of foods. In addition nanotechnologies are being used to enhance the nutritional aspects of food by means of nanoscale additives and nutrients and nanosized delivery systems for bioactive compounds *(background document; section 3.2:Overview of applications).*

A striking observation is that nanotechnologies are being used throughout all phases of food production (Table 1). It has become clear that for applications of nanotechnology in food roughly two classes of application can be distinguished based on the likelihood of consumer exposure to nanoparticles (NPs) or residues of nanotechnologies applications. In the first class, nanotechnology is applied as a production tool, implying that no addition of NPs to the food will take place. Examples of this type of nanotechnology are the use of nanosieves (e.g. to filter out bacteria) or of hand-held devices containing nanotechnology for monitoring purposes. More in contact with food are sensors applied in food packaging materials. In the second class potential consumer exposure to NPs can be expected because NPs are purposely introduced into the food during the production.

Table 1. Summary of applications of nanotechnology in the food production chain

Chain phase	Application	Nanotechnology	Function
Agricultural production	Nanosensors	Nanospray on food commodities	Binds and colors micro organisms
		Hand-held devices	Detection of contaminants etc.
		Incorporated in packaging materials	Detection of food deterioration.
	Pesticides	Nanoemulsions, -encapsulates	Increased efficacy, water solubility and crop adherence
		Triggered release nanoencapsulates	Triggered (local) release
	Water purification/ soil cleaning	Filters with nanopores	Pathogen/ contaminant removal
		Nanoparticles	Removal or catalysation of oxidation of contaminants
Production and processing of food	Food production	Nanoceramic devices	Large reactive surface area
	Refrigerators, storage containers, food preparation equipment	Incorporated nanosized particles, mostly silver, occasionally zinc oxide	Anti-bacterial coating of storage and food handling devices
Conservation	Food products	Nanosized silver sprays	Anti-bacterial action
	Packaging materials	Incorporated sensors	Detection of food deterioration.
			Monitoring storage conditions
		Incorporated nanoparticles	Increasing barrier properties, strength of materials
		Incorporated active nanoparticles	Oxygen scavenging, prevention of growth of pathogens
'Functional food', consumption	Supplements	Colloidal metal nanoparticles	Claimed to enhanced desirable uptake
		Delivery systems "Nanoclusters"	Protecting and (targeted) delivery of content
		Nanosized/-clustered food/drinks (nutrients)	Claimed enhanced uptake

In Annex I an overview of currently available products can be found.

In the second class a diversity of NP types is currently applied in the food production chain, which can be divided in inert particles and nanodelivery systems. Inert particles are used in the food production chain (Table 2) for a variety of purposes. Examples are aluminum oxide, lanthanum particles and nanoscale iron powder in the process of water purification and/or soil cleaning. In food storage, silver

and in rarer cases zinc oxide NPs are applied. Silicate NPs, nanocomposite and silver, magnesium- and zinc oxide are used in food packaging materials. Inert NPs are also processed in food commodities examples are calcium, magnesium, silver, silicate, silicium oxide and white gold NPs. Other applications in food commodities are nanosized particles, regulatory peptides from plants, nanodroplets/- clusters and nanowater (see Table 2). The aim of nanosizing the particles is to increase the bioavailabity of these compounds.It is important to note that the characteristics of abovementioned particles are usually unknown *(background document; section 3.3: Description of types of nanoparticles)*.
Consumer exposure can be expected following direct application of inert particles in the food, while expected consumer exposure is low as long as NPs remain bound in the packaging materials or in the coating on surfaces of packaging materials and food preparation devices. Crucial safety-related issues are migration NP resulting in appearance (e.g. free or as large aggregates) of these NPs in the food. As stated before, especially the free forms of the NPs are reason for safety concern (SCENIHR 2006).
The other type of NPs concerns the nanodelivery systems (Letchford and Burt 2007; Taylor et al. 2005). When incorporated into food the delivery systems are commonly build from peptide or lipid monomers (Chen et al. 2006b; Graveland-Bikker and de Kruif 2006; Mozafari et al. 2006). Examples of these nanoencapsules (see Table 2) range from novel pesticide formulations (e.g. increased crop adherence) to delivery systems for bioactive compounds. These novel formulations may lead to increased human exposure as a result of increased residues in plants. The other major application of encapsulates is incorporation in food (supplements) to deliver bioactive compounds in a targeted fashion and to increase the bioavailability of these compounds.

Table 2: Summary of type of nanoparticles applied in the food production chain

Type of NP	**Application**	**Function**
Colloidal metal nanoparticles	Food additive	Claimed to enhance desirable GI-uptake
Metal nanoparticles (Silver, ZnO)	Food additive/supplement	Claimed enhanced uptake
	Packaging materials/ storage	Increase barrier properties
	Food preparation devices	Clean surface
	Refrigerators, storage containers	Anti-bacterial coating of storage and food handling devices
	Water purification/ soil cleaning	Removal or catalysation of oxidation of contaminants
	Sprays	Anti-bacterial
Nanosized nutrients /foods	Food additive /supplement	Claimed enhanced uptake
Complex nanoscale structures	Nanosensors in packaging	Detection of food deterioration.
		Monitoring storage conditions
	Hand-held devices	Detection of contaminants etc.
Incorporated active nanoparticles	(migration out of) packaging materials	Oxygen scavenging, prevention of growth of pathogens
Filters with nanopores	Water purification	Removal pathogens, contaminants
	Equal emulsions	Product design
Delivery systems (nanoencapsulates)	Food additive / supplement	Protecting and (targeted) delivery of content
	Pesticide	Increased efficacy, water solubility and crop adherence, triggered (local) release

Knowledge gaps

The assessment of potential risks of applications of nanotechnologies in agriculture, like residues in food products, of leakage from packaging materials and of nanoscale food additives, and supplements will require substantial scientific input. There is a lack of knowledge on the exact characteristics of the applied NPs and consequently a lack of knowledge on potential consumer exposure. The wide application of nanotechnology within the food chain will have as a consequence that various regulatory frameworks *(see question 5)* will need to be reviewed for their validity. Regulatory and scientific efforts will have to be carried out both in the light of pre- and post marketing situations.

Research issues/potential impact of research

- **Proper definition of (bio)nanotechnology and nanoparticles applied in food production:** One of the basic problems when discussing safety aspects of nanotechnology is the diversity of nanotechnologies and NPs (e.g. from inert insoluble nanoparticles to delivery systems for pesticides and bioactive compounds). A practical definition will serve as a guide towards prioritization of research as well as towards producers and regulators as a guide for dossier requirements. In addition it is of paramount importance for a transparent discussion with stakeholders and the public *(see question 2).*
- **Overview of type of nanotechnology containing products already on or expected to be introduced on the market.** The advantages of having an accurate overview are discussed under *question 3.*
- **Inventory of scientific requirements for pre- and postmarketing situations:** To adequately assess the safety of products during an authorization procedure or assess the risks of products already on the market, knowledge needs to be gained on various aspects of NPs in food *(see question 6 and question 7).* By gaining more knowledge and experience with respect to NPs the reliability of the current safety and risk assessment will be improved. The inventory itself will be helpful in prioritizing research activities.

2 Is a lack of a strict definition a problem?

The answer can be given easily: it is a clear "yes".
There is a commonly used definition which states that engineered nanoparticles (NPs) are materials that are designed and produced to have structural features with at least one dimension of 100 nanometers or less (Oberdorster et al. 2005a). Thus nanotechnology involves the manufacture, processing and application of materials that are in the size range of 100 nanometers (nm) or less. The size limit once was chosen defined from a more physico-chemical point of view, but not on a toxicological basis.
In international fora like Scientific Committee on Emerging and Newly-Identified Health Risks (SCENIHR) and the International Organization for Standardization (ISO) discussions on definition are high on the agendas. However, most discussions lead in the direction of defining the upper size limit of a NP is as *approximately* 100 nm, which is not strict enough for application in regulatory frameworks.
Another important definition issue is the lack of good metrics to describe a dose of NPs. It has become clear that the currently used metrics for concentration (e.g. mg/kg) are no longer adequate. Up till now it has not been possible to establish an alternative dose-describing parameter that best describes the dose (and the observed dose response relations in toxicological tests). This has led in literature to a general recommendation that NPs used for (toxicological) studies should be characterized as completely as possible (Oberdorster et al. 2005a; Powers et al. 2006; Thomas and Sayre 2005). It has become clear that the size will not be the only critical factor to consider, the total surface area may also be relevant, as well as the number of particles per particle size and perhaps other characteristics *(background document; section 4.1:Physicochemical characterization of nanoparticles).*

Knowledge gaps
The exact size limit of 100 nm in the present definition of NPs is arbitrary due to lack of knowledge on the relationship between particle size, kinetics and toxicological effects. It will be relevant to explore the legal feasibility of avoiding arbitrary size limits, in order to handle the consequences of scientific uncertainties in a more pragmatic way. Such knowledge is not easily derived. Thus, the definition should therefore first be treated in a pragmatic way.
In contrast to conventional[2] chemicals exposure to NPs means exposure to particles that cover a certain range of sizes. Moreover, particles can have a variety of shapes. These two issues already imply that doses cannot be described on a weight or volume basis, but it is also to simple to assume that a one dimensional parameter like surface area can be a good substitute. Probably, multifactorial units, taking into account e.g. the number of particles of a certain size and surface area will need to be developed.

Research issues/potential impact of research
- **Propose a 'working' definition of nanoparticles:** Several international working groups (SCENIHR, ISO) are considering definitions of nanotechnologies and NPs that are adequately describing the novel nature of the NPs and on the other hand are practical from a regulatory point of view. A proper definition, i.e. applicable in regulatory frameworks, will give clarity for both producers and regulators.
  Knowledge on dose-describing parameters can feed these discussions. A proper dose metrics will help researchers to compare study results and will help regulators to formulate health-based limit values. It will also enable risk assessors to compare and combine exposure and hazard information and conclude on the likelihood of health risks.

[2] Convential meaning not nano-sized

3 What products are already on the market?

Food products containing nanotechnologies are penetrating the market, albeit currently predominantly outside the EU (e.g. Japan, China and the USA). It is widely anticipated that they will appear on the EU market in the next few years. Currently many nanoproducts are globally available a.o. due to sales via The Internet. But not all applications and not all nanoparticles (NPs) are alike and thus they do not share the same hazard or risk profile. A ranking of risks given the application and type of NPs should be made. An integrated inventory of applications of nanotechnologies and NPs in food has been made. This inventory has been made using Google™ [3], the database of consumer products of the Nanotechnology project (www.nanotechproject.org) of the Woodrow Wilson International Center for Scholars, in the Global New Products Database of Mintel (www.gnpd.com), the Nanotechnology Product Directory (www.nanoshop.com) and the report of nanoforum (www.nanoforum.org).
The results of this inventory can be found in *Annex I of the background document*. As stated before applications can be found throughout the food production. Products claimed to contain nanotechnology are used in the food processing and storage and applied directly in food commodities (Table 3).

Table 3: Summary of number of products per class of application in the inventory

Class of application	Number of products
Nanosensors	2
Pesticides	5
Water purification /soil cleaning	5
Food processing and storage	10
Food packaging	7
Food commodities: inert particles	9
Food commodities: delivery systems	19
Food commodities: others	9

The number of products per class of application are based on the inventory presented in annex I of the background document.

Knowledge gaps
The inventory is based on labeling information on the product as provided. The claim that these products contain nanotechnology cannot be verified from the information presented. This also applies to the information on the presence and/or type of NPs in these products. It can be expected that the claim 'nanotechnology' on the label of some products is not more than a marketing instrument. Probably even more critical is the fact that products containing nanotechnology or NPs that are not claimed on the labels are for that reason not included in this inventory. Thus instruments needs to be developed for the control of labeling information and validation of databases.

[3] using the search terms 'nano', 'nanotechnology', 'nanotubes', 'nanoparticles', 'food', 'product' in varying combinations

Research issues/potential impact of research

- **Developing an integrated quality-checked database:** The inventory of this project could result in a database existing of nanotechnology containing foods. This database could be extended with a patent database (as developed by DEFRA / CSL in the United Kingdom). Quality of information of overviews on economic perspectives and developments made by consultancy agencies should be evaluated.

  High-quality and reliable databases can be used to obtain a realistic view on products on the market and can thus used for monitoring purposes, priority settings for post-marketing surveys and emerging risk projects.

4 What can be expected to reach the market in the (near) future?

It is difficult to predict accurately the long-term trends of nanotechnology within the agriculture and food industry. Nearby trends will favor those nanotechnologies and application of those NPs that are readily available, for example applications using nano-scale metals, polymers, silica and commonly applied encapsulates. Furthermore, trends in applying nanotechnology in food are likely to be driven by social priority areas to large-value commercial or public sector markets such as human health, agriculture and environment (DEFRA 2005).
Within agriculture, precision farming has been a long-desired goal, making use of smart sensing systems for early warning of e.g. moisture changes, but also nanodelivery systems for pesticides that are able to respond to different conditions. First examples of such applications have been found in the database search (see question 3). Within food industry research on the application of NP in packaging materials aimed at developing smart packages will continue. A development to couple sensing systems to radio frequency identification technology (and thus linking packaging and logistic processes) can be foreseen. While costs of these systems are currently the main drawback, fusions of nanotechnology and electronics should make these transponders cheaper (Nanoforum 2006).
The consumer products databases mention also products that aim at improving the nutritional value of food products. An example is biofortification aiming to reach the most vulnerable, rural poor. Nanotechnology may enhance trace element delivery (M.B. Zimmerman, inaugural speech WUR, 2007).
A next step, that is currently under research, is the development of functional or interactive foods ("on demand" foods), containing nutrients which will remain dormant in the body and deliver nutrients to cells only when needed. A key element is the use of nanoencapsulates (or nanocontainers) in food to deliver nutrients. Products like this, containing nanoencapsulates loaded with nutrients or bioactive compounds, will help to enjoy food but still maintain for example a healthy and or low calorie diet. These novel applications will contribute to the role of foods in preventive healthcare (Kampers 2007).
There is a development of lowering the boundaries between the food and cosmetical domain or between food and pharma, where for example both food and cosmetic industries are developing methods to deliver vitamins to the skin (Nanoforum 2006).

Knowledge gaps
Technological developments and applications of new nanotechnologies and nanoparticles in food will continue. As stated under *question 3* regularly updated quality-checked databases are of high value to obtain a realistic view on products on the market.
Most agricultural and food applications of nanotechnology will be subjected to some form of approval process before a marketing authorization. The adequacy of the current regulatory framework has been reviewed and will be discussed under *question 5.* The general problems identified there will also be relevant for future developments of nanotechnology, e.g. whether food processed at nano-scale should be considered as novel foods. Integration or disappearance of boundaries between types of application (cosmetics, medicines and food) will result in possible aggregated exposure of NPs, consequently this has to be considered in the safety assessment of NPs.

5 Which regulatory frameworks might be involved?

The EU's approach to nanotechnology is 'safe, integrated and responsible' [Eva Hellsten in a Green Week session on 'Future Scenarios for Human Health and the Environment', June 13. 2007]. To that end the EU has commissioned the Scientific Committee on Emerging and Newly-Identified Health Risks (SCENIHR) to make an inventory to check whether nanotechnologies are already covered by other community legislation, thus defining the legislative framework, considering both implementation and enforcement tools for this specific framework. It was concluded that the EU regulatory framework covered in principle also nanotechnologies. The Health Council of the Netherlands considered that: "the best course of action would be to modify existing laws and rules as and when developments within the fields of nanoscience and nanotechnologies render such measures necessary" (HealthCouncilNetherlands 2006). However, it is also clear that implementation of the legal framework remains difficult because of scientific knowledge gaps and fast-evolving market for products.

In this report the most important regulatory frameworks for the authorization of compounds to be used in food have been reviewed:

- The European General Food Regulation (EC/178/2002)
- Novel food [and novel food ingredients] Regulation (EC/258/97)
- Food additives, enzymes and flavorings (89/107/EC; 94/36/EC; 94/35/EC; 95/2/EC and their amendments).
- Food enrichments regulation (EC/1925/2006)
- Food supplements directive (2002/46/EC)
- Food contact materials (EC/1935/2004)
- And regulations and directives on pesticides and veterinary drugs.

Knowledge gaps

Authorization procedures, legislation, guidelines and guidance documents describe how and which toxicity tests should be performed. Adjustments of legislation, guidelines and guidance documents concerning the testing of nanoparticles (NPs) of the substance are considered to be necessary. In particular requirements on information of the physico-chemical parameters, e.g. particle size, particle form, surface properties and other properties that may have impact on the toxicity of the substance, should be included. Furthermore, appropriate dose metrics to use in the hazard characterization and consumer exposure assessments should be developed *(background document; section 5: Review of food related legislation related to nanotechnology in food).*

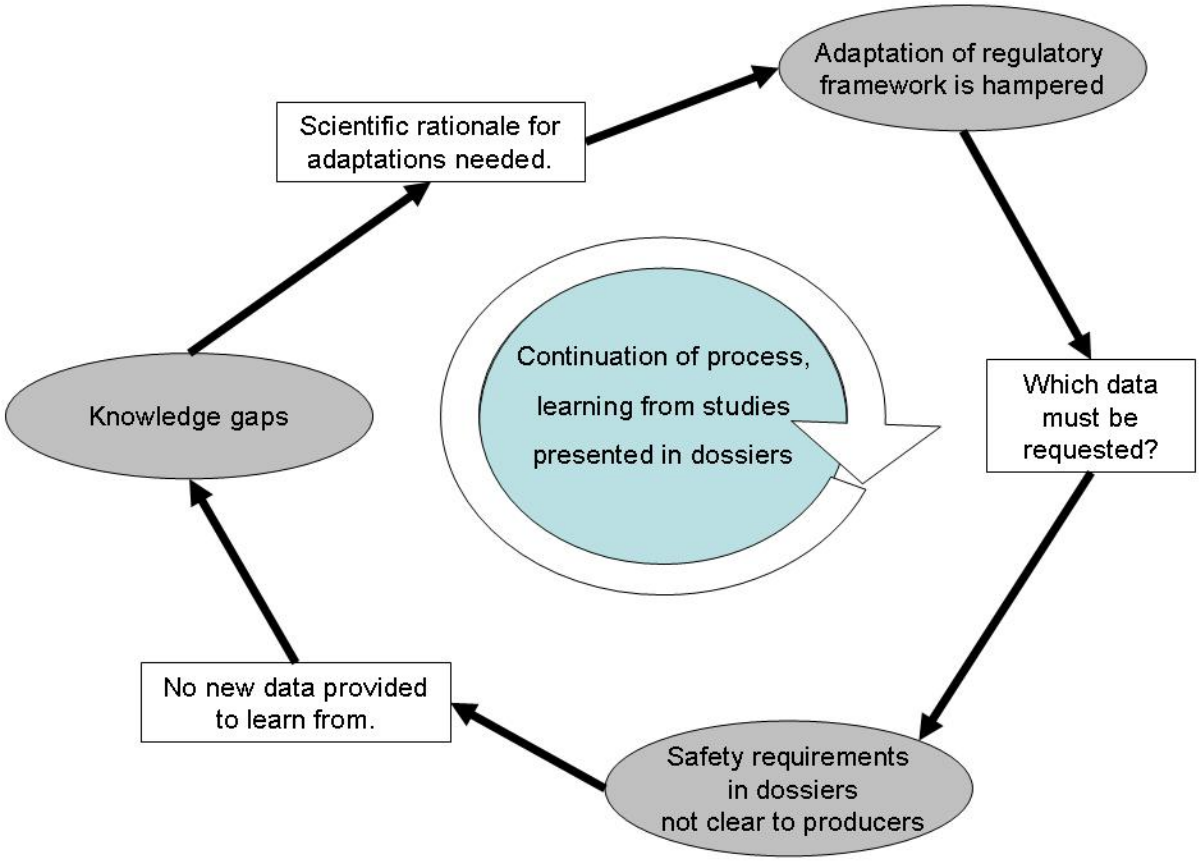
Methodological changes in (OECD) safety test protocols may be required to account for toxicity mechanisms of NPs not found in 'normal sized' materials. Thresholds or limits already set may be not appropriate for nanosized variants of the particular substances.

The review of the regulatory framework demonstrated that the impact of considering nano-sized materials as 'new substances' should be investigated. If a substance in its conventional form has been evaluated, re-evaluation of the nano-sized form may be necessary. One should be aware, that each new nano-sized form of a certain chemical probably has to be considered as a separate new compound, as long as size-effects relationships are not established for that compound. This underscores the need for taking into account the effect of particle size (including distribution of the size) in toxicological studies (see question 7).

The Novel Food Regulation (EC/258/97) can be very relevant for nanotechnology in food. This regulation addresses 'production processes not currently used' making it is likely to assume that this

regulation covers also nanotechnology because of its novelty. It is not clear whether the use of NPs in foods that are already on the market makes these foods 'novel' and thus require authorization. Furthermore the term ‘substantial equivalent’ is introduced. The regulation says that when certain food components are 'substantial equivalent' to their conventional counterparts they can be treated in the same manner as their counterparts. Only the 'equivalency' has to be proven. It is likely that some engineered NPs will be 'equivalent’. The Novel Food Regulation is under revision at this moment, clearly an opportunity to sort out nanotechnology related issues *(background document; section 5.2 : Novel food and novel food ingredients).*

Figure 1. depicts how knowledge gaps affect regulatory frameworks and safety requirements.



Research issues/ Potential impact of research

- **In-depth analysis of relevant regulatory frameworks guidance document and technical annexes:** How adequate is the current legislative system on food safety and novel foods regarding nanotechnologies. This contributes to the discussion whether there is a need for new legislation to deal with the safety aspects of nanotechnologies in food or if guidelines should be adapted to new scientific findings. It will gain insight in the actual adaptations that should be made and will complement the opinion of SCENIHR on the appropriateness of technical guidance documents for new and existing chemicals(SCENIHR 2007). Translation to what their conclusions mean in experimental settings and the relevance for food safety is required at the European level. It is important for both pre- marketing safety assessors as well as for producers to be clear on what is required to be able to convincingly determine the safety of products containing nanotechnologies

- **Legal consultation:** How to interpret legal phrases of the above mentioned EU regulatory frameworks on and consequences of interpretation of legislation. Clarification and unity of interpretation of terminology is important for reasons of transparency and common understanding (between safety assessor, regulators and producers).

6 Does safety testing for nanoparticles require more studies than safety testing for conventional chemical compounds?

Engineered nanoparticles (NPs) can have novel or distinct (toxicological) properties that are attributed to a combination of their small size, physiochemical properties, chemical composition and surface structure (Nel et al. 2006). It is the added functionality of NPs that makes the engineered NPs different from natural small sized particles, but also from their conventional counterparts.
Logically, present safety and risk assessment requirements are based on knowledge gathered for conventional chemicals. Also in these assessment assumptions have to be made because of knowledge gaps. However, uncertainties in these assumptions for example extrapolations from one compound to another are approached on a sound basis of general knowledge. For nanoparticles such a basis is lacking, moreover uncertainties in the safety assessment are expected to be larger (Morgan 2005).

Knowledge gaps
At this stage of (lack of) knowledge of nanotoxicology it is unavoidable that risk assessors need as much information as possible about NPs and their appearance in products. Over time it will be possible to evaluate the data and look for the set of most relevant information. Discussions between product developers, regulators and researchers can already be improved by accepting this as a fact. This request for extra information is not to be considered as a request for extra studies. It can also imply that conventional study approaches need to be redesigned.
The lack of the most optimal dose metrics is an example of a situation where at this moment it is still necessary to gather data on a broad range of physical chemical properties. This will hopefully in future lead to the determination of the set of most relevant data requirements.
The kinetics of nanoparticles may be different compared to conventional chemicals. When there is evidence for uptake, distribution of nanoparticles should be studied more extensively when compared to their conventional counterparts. Of special importance are those parts of the body that are normally protected by barriers like the blood-brain-barrier and placenta. In addition, there are indications that very small particles can intrude in tissues of the digestive tract like the salivary glands, which are not screened in standard toxicological surveys. It is however not clear from which size on it would be relevant to extend the toxicological surveys to extra endpoints. This is discussed further under *question 7.*
If the effects induced by NPs are in general comparable to effects induced by equivalent conventional substances, these will likely be observed in the toxicity studies performed to OECD guidelines. However, if other effects are critical, e.g. effects on organs or tissues that are not routinely studied or physiological disturbances that require specific examination, these effects may not be picked up by standard toxicological testing. To date, it is not known whether the standard toxicological study protocols (e.g. OECD) will be able to detect all specific hazards from NP. This relates to the knowledge gaps identified under *question 7.*
It is clear that in the regulatory framework the responsibility for the safety of the product is assigned to the producers. There currently is a need for guidance on how to approach the safety assessment of NPs, and what information should be presented by producers to the regulatory agencies. To elucidate this a close collaborations between all stakeholders is required.

7 Which safety and risk assessment issues need to be addressed for nanotechnology in food?

Discussions on safety issues of nanoparticles (NPs) and nanotech products can almost entirely be brought back to two, often intertwining, questions:

1. product related questions, e.g. which specific measurements are required in order to come to proper insight into safety of the product.
2. fundamental scientific questions resulting in or based on the development of new conceptual approaches.

Discussions on data requirements and expected performance of current assays have demonstrated that it is important to focus the question on what information is *additionally* required to dossier requirements for conventional chemicals. Some research agendas or roadmaps try to circumvent uncertainties which are accepted in risk assessment of conventional chemicals. Questions like "are *in vitro* tests applicable for NPs" should rather be formulated as "are *in vitro* tests equally applicable for NPs as for conventional chemicals", as the role of *in vitro* test results for chemical in risk assessment is still subject to many uncertainties.
Another important way of focusing the discussion is to keep in mind what will really bring risk assessment to a higher level. In other words, in an area where such an enormous amount of research questions can be/ are raised, it is essential to define those questions that represent the 'needs to know'. This approach should be leading in every kind of roadmap or research agenda that is developed for the field of potential risks of nanotechnology.
A special group of NPs that are applied in food are the nanoencapsulates. The capsules (when applied in food) usually are composed of soft matter, that is generally assumed to be of lower risk than the above mentioned inert particles. In case of the nanoencapsulates safety concerns are mainly related to their function: e.g. to increase the bioavailability of specific bioactive compounds (or pesticides). What are the effects of increased bioavailability of these compounds? The high internal exposure of bioactive compounds as a result of increased bioavailability may lead to toxic effects .
For risk assessment both information on exposure as well as on the (intrinsic) toxicity (hazard) of a compound is required. Determining potential consumer exposure is first of all important to assess the potential risk for consumers. Keeping in mind Paracelcus quote *"Alle Ding sind Gift und nichts ohn Gift; allein die Dosis macht, das ein Ding kein Gift ist"*(All things are poison and nothing (is) without poison; only the dose makes that a thing is no poison). Thus the dose of NPs present in food needs to be determined. As stated earlier, engineered NPs can have novel toxicological properties, that are attributed to their small size, chemical composition and surface structure (Nel et al. 2006). Since it has not been possible to establish a single dose-describing parameter that best describes the toxic effect, NPs should be characterized as completely as possible (Oberdorster et al. 2005a; Powers et al. 2006; Thomas and Sayre 2005). A further complicating factor is that the physico-chemical characteristics of NPs are highly depending on the matrix in which they are present (Oberdorster et al. 2005a; Powers et al. 2006). Thus urging the need to characterize NPs in the food matrix (e.g. *in situ*).

Knowledge gaps

The knowledge gaps cover a wide range of topics which are summarized on the headings below.

- Physicochemical properties of NPs as applied as starting material in the product and as manifested in the final product *(background document; section 4.1: Physicochemical characterization of nanoparticles).*
- Dose metrics in dose response relations: Since it has not been possible to establish a single dose-describing parameter that best describes the possible toxicity, NPs should be characterized as completely as possible (Oberdorster et al. 2005a; Powers et al. 2006; Thomas and Sayre 2005). It is likely that mass is not the good metric (SCENIHR 2006). As long as it is not known which metrics should be used to describe the dose, toxicity tests will have to be analyzed case by case using different dose-describing parameters. It is therefore important for risk assessors to have access to a clear description of the analytical methods that were used to determine the physicochemical properties of the respective NP, to the (raw) experimental data and a sound description of the statistical procedure used to analyze the data *(background document; section 4.1: Physicochemical characterization of nanoparticles).*
- Assessment of exposure:
  - For exposure assessment of nanoscale delivery systems loaded with bioactive compounds or bioactive compounds themselves in nanoscale formulations, both the amount of bioactive compounds at nanoscale or within the capsules as well as the free form in the food matrix has to be determined. For this, the analytical isolation, detection and characterization procedures need to be designed to meet these requirements *(background document; section 4.1: Physicochemical characterization of nanoparticles).*
  - The presence of NP in the food matrix might result in increase bioavailability of substances normally present in the food *(background document; section 4.4: Exposure assessment).*
  - A prerequisite for an exposure assessment is the reliability of the concentration data. The amount and type of NPs, the type of nanodelivery system loaded with bioactive compounds and the amount of bioactive compound in the free from needs to be determined in the food matrix as consumed. It will not always be feasible to measure chemicals and NPs in the food matrix in the consumable form. However, the default or database derived processing factors that are being used for determination of exposure assessment of normal chemicals when the exact effect of processing is unknown, (e.g. pesticides (JMPR)), are not (yet) available for NPs *(background document; section 4.4: Exposure assessment. background document; section 4.5: Risk assessment).*
- Internal exposure: Experimental data so far indicate that novel characteristics of NPs (e.g. size, surface charge, functionalized groups) are likely to influence the absorption, metabolism, distribution and excretion (ADME) (Ballou et al. 2004; des Rieux et al. 2006; Florence 2005; Jani et al. 1990; Roszek et al. 2005; Singh et al. 2006) of NPs present in food. Not much is known of the relationship between these physical-chemical characteristics and the behavior of NPs in the body *(background document; section 4.2: Toxicokinetis of nanoparticles).*
- Adverse effects: Knowledge on the potential toxicity of NPs is limited. Several studies suggest that NPs may have a deviating toxicity profile when compared to their conventional chemical analogues (Donaldson et al. 2001; Nel et al. 2006; Oberdorster et al. 2005a). As mentioned earlier, the question arises whether this different toxicity of NPs can be observed in the standard battery of toxicity tests used in protocol toxicology. It is thought that the standard battery will suffice, but special attention is requested for *(background document; section 4.3: Toxicodynamics of nanoparticles).*
  - **Neurotoxicity**, as results from ADME studies clearly indicate that some NPs can pass natural barriers like the blood-brain barrier (Borm et al. 2006; Silva 2007).

- **Reprotoxicity,** as transfer of NPs across the placenta cannot be excluded, which could lead to embryotoxicity as a result of exposure to NPs (Fujimoto et al. 2005). Data addressing the distribution of NPs to the reproductive cells is, as yet, unavailable. In addition, no clear data showing the distribution of NPs in the fetus are available (Tran et al. 2005).
- **Mutagenicity,** as there are indications that on the cellular level, barriers such as cell membranes do not constitute obstacles for NPs. However, the health implications of such possible interactions are still unknown (Kabanov 2006)UBA 2006). Recently, SCENIHR (SCENIHR 2007) concluded that there is a clear need for validated *in vitro* assays for NP evaluation, including assays with meaningful endpoints for genotoxicity tests*.(background document; section 4.3.6)*
- **Allergenicity (or sensitization).** Even for conventional chemicals much is unknown on the induction of food allergy and the type of exposure required to induce such responses. In the case of NPs this becomes extra prominent for two reasons. First of all it is the possible adjuvant activity of NPs that introduces additional uncertainty. And secondly, because of the actively charged surfaces of NPs it can absorb biomolecules as they pass through the GI tract (Govers et al. 1994)*.(background document; section 4.3.7)*

- **Setting health based guidance values:** The last step in the hazard characterization is the setting of health-based guidance values such as acceptable daily intakes for food additives and pesticide residues. Reference points (e.g. the no-observed-adverse-effect-level or benchmark-dose-level) for the critical effect of a substance form the starting point of the risk assessment. This is a general approach for all substances either being in a conventional form or at a nano-sized scale. It is however still unknown how limit values derived for NP's can be compared to those of equivalent conventional chemicals, due to ongoing discussions on dose metrics *(background document; section 4.5: Risk Assessment).*
- Guidance values are based on toxicological studies performed with NPs with a given bioavailability. NPs are often introduced to enhance the bioavailability of either themselves or of bioactive compounds loaded into them or they may affect the uptake of other nutrients (or contaminants) present in the food. If by some means the bioavailability is changed (increased), this may affect the outcome of the toxicity studies and thus the calculated guidance values. Extrapolation of a health-based guidance value between formulations with different bioavailability might not be possible. Ultimately, this might require setting of separate health-based guidance values depending on the formulation *(background document; section 4.5: Risk Assessment).*

Research issues / potential impact of research

When resolved the formulated research issues should increase the reliability of the current safety and risk assessment of NP in food even within a 5 year period.

- The knowledge gained will help regulators to adapt the regulatory framework properly.
- Development of analytical methods in combination with knowledge on toxicity will be essential for upholders
- Reduction of present uncertainties will help to gain the public's trust for this technology and its products.

- **Physicochemical properties and stability in the product matrix:**
  - At present there is a vast array of analytical techniques to characterize NPs (Oberdorster et al. 2005a; Powers et al. 2006; Thomas and Sayre 2005). Often the physicochemical characterization requires a well-equipped laboratory. Literature on isolation of NPs from biological or food matrices is scarce as is the literature on in situ detection methods. Every dilution, extraction or cleaning procedure may affect the appearance of the NPs and result in an incorrect measurement of the NP in the matrix. Potentially this will have great impact on the safety assessment of NPs. It may lead to both false-positive or false-negative conclusions regarding potential exposure to NPs.
    Therefore research should focus on methods that are able of *in situ* detection and characterization of NPs, and that are relatively easily performed with apparatuses that are currently present at laboratories suited for detection of chemicals in food. Ideally, isolation and characterization methods should be developed, suitable for routine and low-cost analysis.
  - It is important to known which additional information regarding physicochemical properties (more than currently presented in dossier of conventional chemicals) will be needed in dossiers for an assessment of NPs in products.
  - A special case might be the NPs used in packaging materials. Current migration assays for chemicals will need to be evaluated for their validity in measuring the migration of NPs from the packaging material into the food.
  - Selecting the matrix in which the NP needs to be characterized is not an easy choice. The matrix should reflect the potential consumer exposure to a NP - food product as accurately as possible.
- **Dose metrics:**
  - It has up to now not been possible to establish a single dose-describing parameter that best describes the (toxic) effects. A pragmatic basic set of characteristics should be developed that describes the dose well enough, e.g. size and size distribution and/or total surface area, and is also practically feasible with respect to analytical requirements. It is important to keep in mind that a dose of NPs contains a range of sizes of a certain type of NPs. This implies that information on mean particle size is not sufficient to describe a dose properly. Moreover a conceptual model for the most optimal unit describing a dose and based on a combination of physics, basic chemical characteristics and toxicological findings should be further developed.
- **Internal exposure:**
  - The validity of currently existing *in vitro* model systems for the gastrointestinal absorption needs to be studies.
  - When there is evidence for gastrointestinal absorption of nanoparticles, distribution to a wide range of tissues should be studied (including the liver, spleen, kidneys, bone marrow, lungs and brain). Keeping in mind that generally only a few tissues and organs are examined in guideline kinetic (OECD) studies. The same holds true for the use of nanoencapsulates aiming at targeted delivery of bioactive compounds. Special attention is required in case of (increased) bioavailability and distribution to tissues that are normally protected by biological barriers such as the blood-brain barrier.
  - Furthermore there is a need for fundamental research on the absorption, distribution, metabolism and excretion (ADME) of NPs to elucidate the driving forces and mechanisms behind these processes. This would greatly facilitate the extrapolation and modeling approaches. However, if the current ADME studies are performed with adequately characterized NPs and a wide range of tissues are analyzed when there is evidence for systemic uptake sufficient information would become available for a reliable ADME assessment.

- Due to the potential impact on toxicological effects special attention needs to be paid to observations that certain NP can cross the blood-brain barrier and the data lack on potential for crossing the placenta.
- Special attention is required for cellular kinetics in order to better understand and predict cellular toxicity and the validity of currently used *in vitro* models.

- **Adverse effects:**
  - **Neurotoxicity** needs to be considered carefully when there is evidence for NP passage of the blood-brain barrier. Risk assessors should be aware of possible neurological effects when assessing toxicology experiments. Possibly, current guideline tests will need to be adapted to render these tests more sensitive for neurotoxic effects of NPs.
  - **Reprotoxicity and embryotoxicity** needs to be considered carefully when there is evidence for NP passage of the placenta. This is not only relevant for inert NPs but also for bioactive compounds that are loaded within nanoencapsulates.
  - **Mutagenicity.** Develop and validate *in vitro* assays for the gastero-intestinal tract. Many NPs have in common to trigger the release of reactive oxygen species and cause oxidative stress by means of interaction with the reticulo-endothelial system (Donaldson et al. 2007; Nel et al. 2006). Model systems for testing genotoxic potential should therefore be a combination of gut derived cell lines and cells from the reticulo-endothelial system (e.g. macrophages). Knowledge on the use of the outcome of *in vitro* assays and profiling studies for risk assessment needs to be developed further.
  - **Allergenicity (or sensitization).** The special role of NPs in developing food allergy needs to be studied. The possible adjuvant activity of NPs are amongst others a reason for serious concern. If a relation between food allergy and a NP is established, traceability is considered to be critical to anticipate and exclude possible sources for such potential allergens (Kroes et al. 2002).
- **Exposure assessment:**
  - Investigate whether the default or database derived processing factors for exposure assessment of conventional chemical needs adaptations for NPs.
- **Other:**
  - What is the feasibility of labeling of products.
  - Additional effort is needed for the education of nanoparticle/ nanotechnologies risk assessors, since this requires a very broad scope of expertise, which is particularly challenging given the rapid scientific developments in the emerging field of science.
  - How to communicate about potential risks.

8 Different safety issues for pre- and postmarketing nanotech products?

The survey of products containing nanoparticles (NPs) indicated that a wide variety of products is on the market, especially via sales on the Internet, that likely contain nanotechnology or NPs. Part of these product are subjected to pre-marketing safety assessments. This means that, depending on the regulatory framework, a dataset of standard toxicological studies and an assessment of health risks need to be submitted for the application of the substance/NP in food. In a safety assessment made prior to market introduction of a substance it is important to address the special physicochemical features of NPs (for a NP as such and the NP in the food matrix), their intrinsic hazards (*hazard identification*), dose-response(effect) levels and kinetic properties (*hazard characterization*) and potential intake levels *(exposure assessment)*. In general this results in an integrated safety assessment and the establishment of acceptable intake levels for humans (*Risk characterization)* and this can form the basis for the definition of necessary maximum use levels or maximum residue levels in food by risk managers (*Risk management*).
It is clear that a wide range of products are available via internet, especially products in the category of the food supplements and food additives. This is a global market, where European consumers can purchase products directly from everywhere around the world. It can be argued that it will be very difficult for national authorities within the EU to strictly enforce EU regulations on this market. This makes it very likely that consumers can expose themselves to products of which the safety is by no means guaranteed. This requires a post marketing risk assessment framework to be in place.

Knowledge gaps

- **Adequateness of guideline toxicological studies and risk assessment methodology**. From the legal requirements imposed by the application of a substance (NP) in food, toxicological studies have to be performed and submitted to provide insight in the possible adverse effects of NP. Given the uncertainties identified under *question 7*, it cannot be concluded yet whether the study protocols for existing guideline toxicological studies will be able to detect all effects of NP.
- **Adequateness pre-marketing data requirements**. Since it is not known whether the current guideline studies are adequate to detect the possible effects of NP, it is also not possible to judge whether the present legal data requirements are adequate (see question 7).
- **Availability of data on nanotechnologies containing products already on the market**. Data on market penetration of NP containing food products and the consumer use of food containing NP is currently not at hand (see question 1 and see question 3).

Research issues/ potential impact of research

- **Adequateness of guideline toxicological studies and risk assessment methodology**. Make an more detailed overview of relevant dossier requirements, to indicate what information should additionally (or not) be requested for NP compared to conventional chemicals *(see also question 5).* New or other legal requirements for pre-marketing safety assessment of NP for application in food can then be developed, guidance to producers can be provided. Adaptation of existing or development of new protocols for testing of toxicological effects of NPs may be a result.
- **Adequateness pre-marketing data requirements**. Once more information is available on the dose metrics, health effects of and exposure to NP in food, the adequateness of the pre-marketing safety research should be assessed.
- **Availability of data on nanotechnologies containing products already on the market**.. The development of an integrated database as identified under *question 3* is important. In addition

monitoring of consumer use of food containing NP is relevant. High quality and reliable databases can be used to obtain a realistic view on products on the market and thus used for monitoring purposes, priority settings for post-marketing surveys and emerging risks projects. The post-marketing surveys should provide detailed information on the market penetration and type of nanotechnology applied in products.

9 How to come to the most efficient research approach?

Various research agendas and roadmaps have been defined for the domain of human health and environmental risks of nanotechnology (OECD, (Maynard et al. 2006), EU, NNI, …). They were developed on the basis of various scopes, from a product point of view, from a more fundamental research point of view, from an economic point of view. Overall they have led to more or less the same research items, that are defined at a quite high level of abstraction. Moreover, a lot of these roadmaps were developed on a scientific or on a regulatory/policy basis. To our opinion, such roadmaps are best developed by an interaction of researchers, policy makers/upholders, and other stakeholders. It is important that all stakeholders have the same goal in mind, i.e. the development of 'responsible' nanotechnology products.

Starting points:

- While stimulating the economic developments of nanotechnologies the safety for human health and the environment may not be compromised.
- Safety research should contribute to the sustainable development of nanotechnologies (used in the food production chain).
- Products have already come to market, so first attention should be paid to post-marketing risks.
- Risk assessment requirements and not fundamental toxicological issues should be leading in developing roadmaps for research in the most efficient way.

For consideration:

- Identify which areas for food in nanotechnology are important for the Netherlands or the EU. The questions raised and research topics mentioned are global issues.
- Identify how research efforts relating to post-marketing risks should weigh in comparison to pre-marketing risks.

10 How can research issues for nanofood safety be prioritised ?

What can be leading issues in prioritizing research items?
At this moment already a large variety of products is at the market or is expected to reach the market within the near future. This implies that in the first place:

1. research should be carried out that supports post marketing risk assessment.
2. the current regulatory framework should be adapted in such a way that products expected in the near future are covered by a relevant regulatory framework.

The conclusions seem to be obvious but it will still be difficult to translate this in concrete research proposals. The following research topics are considered to contribute pivotally to risk assessment of nanotechnologies and nanoparticles in general and thus also for applications in food products.

- *Characterization of nanoparticles.* The particles have novel properties compared to conventional chemicals. It is important to characterize these properties to enable realistic estimations of consumer exposure. But equally important, this information is needed to establish dose-response relations in toxicology studies. Thus, analytical tools need to be developed for the isolation and characterization of nanoparticles in food and biological matrices.
- *Dose metrics.* This is a very basic issue which affects both interpretation of scientific studies as well as regulatory frameworks. It has become clear that doses of nanoparticles and thus also limit values for nanoparticles cannot be expressed in weight or volume measures as is the case for conventional chemicals. Questions arise whether nanosized particles of their conventional counterparts need their own limit values.
- *Effects of nanoparticles.* The kinetics of nanoparticles may be different compared to conventional chemicals. When there is evidence for uptake, distribution of nanoparticles should be studied more extensively when compared to their conventional counterparts. Of special importance are those parts of the body that are normally protected by barriers like the blood-brain-barrier and placenta.
- *Definition of nanoparticles.* This is not only a formal issue for regulators but also very important for discussion on prioritization of research and exchange of study results between scientists, producers and regulators.
- *Consumer exposure to nanoparticles.* It needs to be studied which products containing nanoparticles are on the market and which type of particles are used, and are being developed.

Specifically for applications of nanoparticles and nanotechnologies in food products are the following issues thought to be relevant:

- *Oral bioavailability*
- *Measurement of nanoparticles in food matrices*

Part B.

Health impact of nanotechnologies in food production:

Background document

1 Introduction

The potential benefits of nanotechnology have been recognized by many industries, and products based on nanotechnology or products containing nanoparticles (NPs) are already manufactured such as in the field of electronics, consumer products and pharmaceutical industry. Achievements and discoveries in nanotechnology are beginning to impact the food associated industries (Chen et al. 2006a). Nanotechnology is a new and fast emerging field that involves the manufacture, processing and application of materials that are very small in size. Engineered NPs are commonly defined as materials designed and produced to have structural features with at least one dimension of 100 nanometers or less (Oberdorster et al. 2005a). NPs can be spherical, tubular, irregularly shaped, or can exist in fused aggregated or agglomerated forms. Due to novel physiochemical properties of engineered NPs that are attributed to their small size, chemical composition and surface structure, NPs can have novel or distinct (toxicological) properties (Nel et al. 2006).
Within the food production nanotechnology tools are used in the entire food chain e.g. during cultivation (agriculture), industrial processing or packaging of foods. In addition nanotechnologies are being used to enhance the nutritional aspects of food by means of nanoscale additives and delivery systems added to the food. Various types of NPs can be employed within food industry ranging from inert types of NPs like nanofibers, metal and metal-oxides, quantum dots (Hardman 2006) and other NPs, to delivery systems like liposomes and other forms of nanocapsules (Taylor et al. 2005). Household use may include the use of nanocoated/treated containers for food storage or household appliances.
Generally there is good appreciation of the potential benefits of nanotechnology for the food industry and likely the consumer. However, not too much is known on safety aspects of the application of nanotechnologies in food production and of the incorporation of NPs in food products. Not all applications and not all NPs are alike and thus they do not share the same hazard or risk profile. A ranking of risks given the application and type of NPs should be made, In its evaluation of potential health risks of products containing nanotechnology SCENIHR stated that "… The situation with free nanoparticles, including agglomerates, is quite different. …free nanoparticles [that] give rise to concerns over possible human health and environmental risks" (SCENIHR 2006).

1.1 Aim of the project

The aim of the project is to identify knowledge gaps in the expertise needed to make reliable risk assessments for human health risk in case of application of nanotechnology in food production. Based on this identification of knowledge gaps a priority list of research questions will be drafted, when resolved the answers will contribute to reduce the uncertainties in safety and risk assessments of NPs in food products and the use of nanotechnology in food production within a few years. This part of the report can be found in part A of this report.

1.2 Outline of project

Firstly, an overview of current (or in the near future foreseeable) applications of nanotechnology and NPs in food production will be provided. State-of-the-art of applications of nanotechnology within the following areas will be presented:

- residues of use of nanotechnologies and NPs during production and processing
- packaging materials

- food additives and nutrients

This exploratory task mainly relies on some international inventories from the Woodrow Wilson International Center for Scholars and the European Nanotechnology Gateway to which information from a literature search will be added.
Secondly, the safety assessment performed in the pre-marketing (authorization procedure) assessment of NPs or new products containing NPs will be evaluated. Specific (dossier) requirements resulting from the novel features of the NPs will be highlighted. However data requirements for conventional forms of chemicals also applicable for NPs will not be addressed. Most important knowledge gaps at each step of the safety assessment will be identified and accompanied by a project proposal. If executed these projects should increase the reliability of the safety assessment (e.g. hazard identification, hazard characterization, exposure assessment and risk characterization). Additionally some remarks on the post marketing risk assessment will be provided.
Thirdly, a general overview with the Dutch point of view on the legal framework of nanotechnology application within the food area will be provided.
Results will be used to provide the risk manager with an identification of uncertainties in the current risk assessments.
The synthesis report gives an overview and an advice for priority of scientific issues that needs to be addressed in order to improve the process of post-marketing risk assessment for NPs in food and in order to gain insight in dossier requirements for NPs in food.

1.3 Out of scope

This project only covers health impact of application of nanotechnologies during food production and/or for consumers. Potential health impact of application of nanotechnologies in other industries e.g. electronics, medical and consumer products will not be addressed in this project.

2 Nanotechnology - definition and applications

The Nobel Laureate Richard Feynman is suggested to be the first person to have the vision to see the potential of working at the nanoscale. In a visionary presentation in 1959 titled "There's plenty of room at the bottom", he postulated that being able to manipulate atoms and molecules at will would open up new avenues of technology (Feynman, 1959). Working at the atomic level became only within reach when key analytical tools like the scanning tunneling microscope were developed in the 1980s. Advances like these and other analytical tools quickly spread to be utilized in many other fields of science. This led to the development of materials showing unique properties that are dependent on their nanostructure c.q. size. Current research is leading to the development of sophisticated and heterogeneous materials and devices, based on an increasing ability to engineer in functionality at the nanoscale (Roco 2004). In his review of the development of nanotechnology Maynard emphasizes that the benefits that have the potential to change and improve our lives will inevitably bring with them new risks that need to be identified and managed (Maynard 2006).
Nanotechnology itself and its applications are now rapidly growing, hundreds of claimed nanotechnology products from enhanced materials, electronic products and devices and pharmaceutical products are already on the market (The Nanotechnology Consumer Inventory, 2006). It is this broadness of application of nanotechnologies that makes it particularly difficult to discuss potential risks in general terms. Moreover, the broadness also makes this technology very sensitive for consumer anxieties, because (negative) discussions on applications nanotechnologies in one sector will have its effect on applications in another sector.
As nanotechnology is an enabling converging technology used in many industries a discussion on the risks must start with a definition and a focus on the field of application.

2.1 Definitions of nanotechnologies and nanoparticles

Nanotechnologies are enabling and converging technologies, which means that it is not a single type of technology used in a single field of science, but a great variety of techniques that have only one thing in common: the size-scale:

Nanotechnologies: The design, characterization, production and application of structures, devices and systems by controlling shape and size at the nanometer scale (Engineering. 2004)
The Royal Society purposely uses the plural of nanotechnology, to express the diverse range (and applications) of nanotechnology.
Other groups have included an extra criterion: it is not only the small size that matters, but also the added novel characteristics or properties of the new substances, products and applications that makes nanotechnologies a special group of technologies:

> *Nanotechnology: The understanding and control of matter at dimensions of roughly 1 to 100 nanometers, where unique phenomena enable novel applications (NSET, 2004)*

Nanoscience: The study of phenomena and manipulation of materials at atomic, molecular and macromolecular scales, where properties differ significantly from those at a larger scale ((Engineering. 2004)

This was reworded by Maynard (2006), in his view nanotechnologies have three things in common:

- *Control – the ability to put small quantities of matter where it is wanted.*
- *Utilization – using this ability for some practical purpose.*
- *Visualization – detecting where material is placed and how it is configured at the nanoscale (Maynard 2006)*

The development and application of nanoparticles (NPs) is one of the results of the use of nanotechnologies. Besides engineered NP, nanosized particles also can have a natural origin like sand dust, ashes as a result of volcano eruption, or can be the unintended result of human activities. Examples of the latter are ultra fine particles in diesel fumes (combustion). These non-engineered particles have been studied extensively, especially the toxicity due to inhalation of these particles.

In summary the novel (unusual) properties of engineered NP are attributable to their (Nel et al. 2006):

- *small size: resulting in a relatively large surface area (and distribution in sizes).*
- *chemical composition: purity, crystallinity, electronic properties etc.*
- *surface structure: surface reactivity, surface groups, inorganic or organic coatings, etc.*
- *solubility, shape and aggregation.*

Within the food production industry it can be envisaged that engineered NPs are or will be applied in packaging materials, processing aids or as food additives. A different and in food area very important type of NPs are delivery systems like liposomes and other forms of nanocapsules (Taylor et al. 2005). Encapsulation will be used in novel formulations of pesticides and veterinary drugs, or to enhance the controlled release of food ingredients at the right place and the right time (Gouin 2004).

In this document the term ‘nanoparticle’ is used as a general denominator for various types of engineered NPs that have at least one dimension on the nanoscale, e.g. between 1 and 100 nm, and that are ‘fixed’ or ‘free’:

- Fixed: Nanoscale patterning at the surface, or nanocomposites in which NPs are permanently embedded into a conventional matrix structure are examples of fixed NPs. The interactions of fixed NPs with living systems is limited by the fact that these NPs cannot be taken up into tissues or individual cells. Fixed NPs can be released from their matrix (e.g. wear off, migration etc)
- Free: Free NPs have two (nanorods, nanowires) or three of their dimensions in the nanoscale, and as such are small enough to be taken up into individual cells. This opens up new possibilities for interactions between NPs and cells, which may result in alterations in cellular signaling and cell function.

Furtheron in this report with nanoparticles only the nanoparticles in its free form are discussed.

3 Applications of nanotechnologies in the food production chain

Nanotechnology has the potential to impact many aspects of food and agricultural systems. Bioavailability, food security, disease treatment, delivery systems, new tools for molecular and cellular biology, new materials for pathogen detection are examples of the important items that are linked with nanotechnology (Weiss et al. 2006).

3.1 Approach

In this section an overview of products made using nanotechnology or nanoparticles (NPs) are applied is provided. The inventory has been made using Google™, using the search terms 'nano', 'nanotechnology', 'nanotubes', 'NPs', 'food', 'product' in varying combinations. Most products have been found via the database of consumer products of the Nanotechnology project (www.nanotechproject.org) of the Woodrow Wilson International Center for Scholars, in the Global New Products Databse of Mintel (www.gnpd.com), and the Nanotechnology Product Directory (www.nanoshop.com) and the report of nanoforum (www.nanoforum.org). The databases and the internet have been searched during Spring 2007.

3.2 Overview of applications

The overview of the products that are claimed to be produced using nanotechnologies or containing NPs is provided in Annex 1**.** The results are summarized in Tables 3.1 and 3.2.
The inventory clearly indicates that nanotechnologies are being used throughout all phases of food production (Table 3.1). It is striking that it is not one type of technologies that is being used: it ranges from processing techniques to the application of 'inert' or encapsulate NPs into food products.

Before analyzing the results of the inventory, this search needs some critical discussion. The inventory is based on labeling information on the product. The claim that these products contain nanotechnology cannot be verified from the information presented. This also applies to the information on the presence and/or type of NPs applied in these products. It can be expected that the claim 'nanotechnology' on the label of some products is not more than a marketing instrument. On the other hand products containing nanotechnology or NPs that are not claimed on the labels are for that reason not included in this inventory. The results of this inventory are thus clearly biased.

Types of application
The most striking observation is that nanotechnologies are being used throughout all phases of food production (Table 3.1). It is clear that it is not one type of technologies that is being used: it ranges from nanoformulated agricultural compounds (e.g. pesticides), to processing techniques and the addition of inert or encapsulate NPs into food products. The type of application can be used as a first estimate of potential consumer exposure and thus as a ranking of risks. Nanotechnology used for the food production without introducing (e.g. adding) nanoscale products or compounds in the food like filters with nanopores, can be considered of low risk for the consumer. The use hand-held devices containing nanotechnology or filters with nanopores to create monodisperse solutions or to filter out microbiological contamination are examples of this category of applications.

Direct (intentional) application of NPs in food can start early in the food chain when nanoformulations of pesticides are applied directly on crops. The other potential source might origin from residues of NPs for water and soil cleaning purposes in fields on which crops are cultured, could become available for the consumer. Or by contamination with NPS as a result of environmental release of NPs from production processes.
In addition, the application of 'sensor sprays' to conventional materials to monitor contamination with micro-organisms is a direct source for NPs in food when products from the surface are consumed. While filters with nanopores (and therefore using nanotechnology) become in direct contact with food this application of nanotechnology is expected to have no additional safety concerns in comparison with conventional filters. The type of material (and wear-off as result of use) of the filters or cleaning products of the filters might require some attention, but this is clearly not exclusively related to safety of nanotechnology.
The ultimate direct consumer expose can be expected when NPs are included into food directly (with an e.g. antimicrobial-, enhancing bioavailability- or targeted delivery function).
Indirect application of NPs in food can be expected when nanotechnological devices are incorporated in packaging materials, storage containers of food preparation equipment. In packaging materials two types NP can be identified. Inert particles to increase barrier or strength properties of the packaging materials and (re)active particles. The latter are designed to respond to environmental changes (e.g. temperature in storage rooms), degradation products of the food commodities, or contamination by micro-organisms. NPs used in these applications can migrate or wear-off from the materials before consumers exposure can be expected.

Inert particles are used in the food production chain (Table 3.2) with a diverse aimed function. Examples are aluminum oxide, lanthanum particles and nanoscale iron powder in the process of water purification/ soil cleaning. In food storage and processing, silver and zink oxide NPs are handled in refrigerators and storage containers. Silicate NPs, nanocomposite and magnesium - and zink oxide are used in food packaging materials, while also inert particles are processed in food commodities, like calcium, magnesium, silver, silicate, silicium oxide and white gold. Other applications in food commodities are nanosized particles, regulatory peptides from plants, nanodroplets/- clusters and nanowater. However, the form of abovementioned particles is usually unknown. Consumer exposure can be expected following application of inert particles in the food, while expected consumer exposure is low as long as NPS remain bound in the packaging materials or in the coating on surfaces of packaging materials and food preparation devices (risk of wea-off). There, crucial safety related issues are migration of these in to food and appearance (e.g. free or as large aggregates) of these NPS in the food. As stated before, it are the free forms of the NPS that are reason for safety concern (SCENIHR 2006).
The other type of NPS that are encountered are the nanodelivery systems, of which a diversity of forms exist (Letchford and Burt 2007; Taylor et al. 2005). When incorporated into food the delivery systems are commonly build from peptide or lipid monomers (Chen et al. 2006b; Graveland-Bikker and de Kruif 2006; Mozafari et al. 2006). These Nanoencapsules are used for novel pesticide formulations, consumers exposure to residues of these particles can thus be expected. The other major application of encapsulates are the use of delivery system for bioactive compounds, e.g. to increase the bioavailability of these compounds.

While potential consumer exposure might be a useful approach to ranking potential risks of nanotechnology, this could also be approached from a regulatory side. Which applications are likely adequately subjected to a regulatory framework and what is the likelihood of enforcement? All compounds that are to be used in food or expected to be used in products in contact with food are subject to pre marketing authorization procedures. Most import regulatory frameworks for the authorization of compounds to be used in food are the following:

- The European General Food Regulation (EC/178/2002)
- Novel food [and novel food ingredients] Regulation (EC/258/97)
- Food additives, enzymes, flavourings and processing aids (89/107/EC; 94/36/EC; 94/35/EC; 95/2/EC and their amendments).
- Food enrichments regulation (EC/1925/EC)
- Food supplements directive (2002/46/EC)
- Food contact materials (EC/1935/2004)
- And regulations and directives on pesticides and veterinary drugs.

In general terms these regulations have safety provisions incorporated. It is the producer's responsibility that their products are safe. Regulatory agencies assess the dossiers that are submitted by applicants. The adequacy of the safety provisions and testing will be elaborated on in the following sections. However, it is also clear that a wide range of products, especially the food supplements and food additives, are available via internet.. While all products on the (internet) market in principle are subjected to the above indicated regulatory framework it can be argued that it will be very difficult for national authorities to strictly enforce this market. This will necessitate a post marketing risk assessment framework to be in place.

Table 3.1 Summary of applications of nanotechnology in the food production chain

Chain phase	Application	Nanotechnology	Function
Agricultural production	Nanosensors	Nanospray on food commodities	Binds and colors micro organisms
		Hand-held devices	Detection of contaminants etc.
		Incorporated in packaging materials	Detection of food deterioration.
	Pesticides	Nanoemulsions, -encapsulates	Increased efficacy, water solubility and crop adherence
		Triggered release nanoencapsulates	Triggered (local) release
	Water purification/ soil cleaning	Filters with nanopores	Pathogen/ contaminant removal
		NPs	Removal or catalysation of oxidation of contaminants
Production and processing of food	Food production	Nanoceramic devices	Large reactive surface area
	Refrigerators, storage containers, food preparation equipment	Incorporated nanosized particles, mostly silver, occasionally zinc oxide	Anti-bacterial coating of storage and food handling devices
Conservation	Food products	Nanosized silver sprays	Anti-bacterial action
	Packaging materials	Incorporated sensors	Detection of food deterioration.
			Monitoring storage conditions
		Incorporated NPs	Increasing barrier properties, strength of materials
		Incorporated active NPs	Oxygen scavenging, prevention of growth of pathogens

Chain phase	Application	Nanotechnology	Function
'Functional food', consumption	Supplements	Colloidal metal NPs	Claimed to enhanced desirable uptake
		Delivery systems "Nanoclusters"	Protecting and (targeted) delivery of content
		Nanosized/-clustered food/drinks (nutrients)	Claimed enhanced uptake

Table 3.2 Summary of type of NPs and NPs applied in the food production chain

Type of NP	Application	Function
Colloidal metal NPs	Food additive	Claimed to enhance desirable GI-uptake
Metal NPs (Silver, ZnO)	Food additive/supplement	Claimed enhanced uptake
	Packaging materials/ storage	Increase barrier properties
	Food preparation devices	Clean surface
	Refrigerators, storage containers	Anti-bacterial coating of storage and food handling devices
	Water purification/ soil cleaning	Removal or catalysation of oxidation of contaminants
	Sprays	Anti-bacterial
Nanosized nutrients /foods	Food additive /supllement	Claimed enhanced uptake
Complex nanoscale structures	Nanosensors in packaging	Detection of food deterioration. Monitoring storage conditions
	Hand-held devices	Detection of contaminants etc.
Incorporated active NPs	(migration out of) packaging materials	Oxygen scavenging, prevention of growth of pathogens
Filters with nanopores	Water purification	Removal pathogens, contaminants
	Equal emulsions	Product design
Delivery systems (nanoencapsulates)	Food additive / supplement	Protecting and (targeted) delivery of content
	Pesticide	Increased efficacy, water solubility and crop adherence, triggered (local) release

3.3 Description of types of nanoparticles

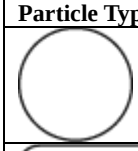
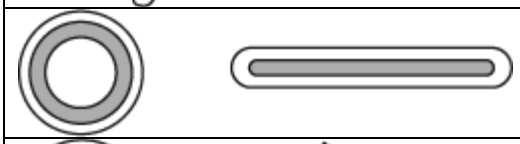
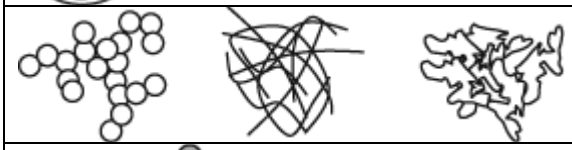
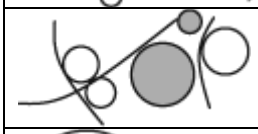
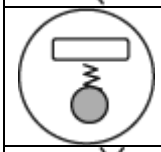
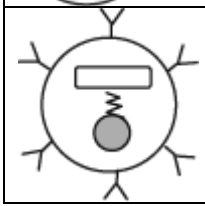
3.3.1 Inert particles

A NP consists of a solid or liquid nanostructure present in the air as an aerosol (mostly solid or liquid phase in the air), a suspension (mostly solid in liquids) or an emulsion (two liquid phases). Different characteristics of NPs are:

- particle size
- surface area per unit mass
- shape
- solubility and dissolution
- reactivity
- coagulation or aggregation state
- chemical composition
- other

As can be seen also from Table 3.3, NPs can be spherical, tubular, irregularly (non-spherical) shaped, or can exist in fused aggregated or agglomerated forms.

Table 3.3 Different types of nanostructured particles

Particle Type	Description
	Spherical or compact particles Compositionally homogeneous
	High aspect ratio particles Compositionally homogeneous
	Complex non-spherical particles Compositionally homogeneous
	Compositionally heterogeneous particles Core-surface compositional variation
	Compositionally heterogeneous particles Distributed compositional variation
	Homogeneous agglomerates Agglomerates of a single particle class
	Heterogeneous aggregates Aggregates of diverse particle types
	Active particles Particle behavior and properties depend on external stimuli
	Multifunctional particles Particle behavior and properties depend on functional responses to local environment and stimuli

These characteristics are important with respect to potential risks for health or environment and determine their fate and behavior in the environment, humans and other organisms.

3.3.2 Encapsulates

Nanoencapsulation involves the incorporation, absorption or dispersion of bioactive compounds in/at or on small vesicles with nano (or submicron) diameters that may protect the bioactive compounds against degradation, improve the stability and solubility of the substance and therefore increase the bioavailability and delivery to cells and tissues (Letchford and Burt 2007; Taylor et al. 2005). Reducing the size of the encapsulates into the nanoscale offers opportunities related to prolonged gastrointestinal retention time caused by bio-adhesive improvements in the mucus covering the intestinal epithelium (Chen et al. 2006b; Medina et al. 2007). Modulations of surface properties (e.g. coatings or biomolecular flags) can enable targeted delivery of compounds. The latter field of application is typical of biomedical application of encapsulates.

Nanoencapsulates may consist of a core composed of one to several types of compounds surrounded by a wall or barrier (see fig 3.1. for types of delivery systems relevant for food industry). These delivery systems have its roots in the pharmaceutical industry where often synthetic polymeric nanoencapsulates are employed. For application of nanoencapsulates into food, lipid- or natural polymers-based capsules are most often applied or studied (Chen et al. 2006b). However, one of the main problems with these natural polymers is the stability of the nanoencapsulates.

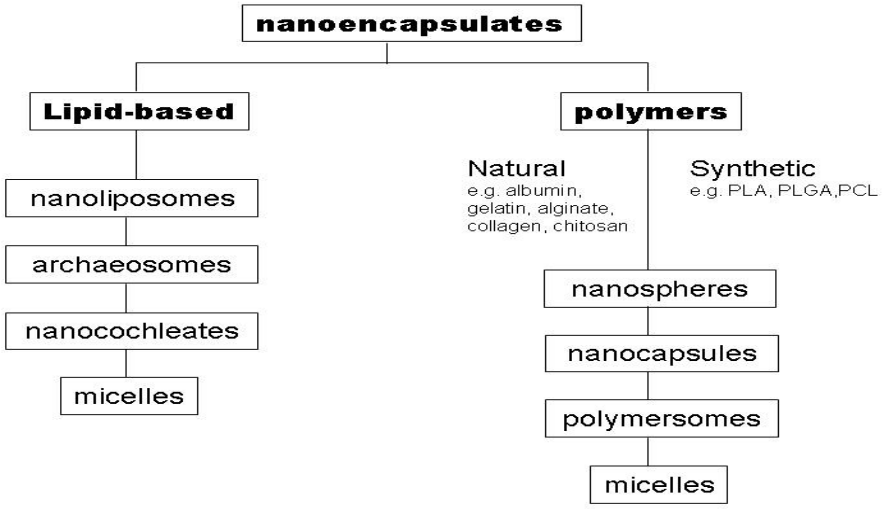


Figure 3.1 Classification of nanoparticulate delivery systems.

Lipid-based nanoencapsulation:
The main lipid-based nanoencapsulation systems that can be used in food and food supplements are nanoliposomes, archaeosomes and nanocochleates (Mozafari et al. 2006).
- Nanoliposomes are defined as bilayer lipid vesicles possessing and maintaining nanometric size ranges during storage and application. Because of their unique properties they can entrap, deliver and release both water-soluble and lipid-soluble material (Mozafari et al. 2006). Liposomes may release their contents into cells upon e.g. encountering specific cellular enzymes, due to pH or thermo-sensitivity or after antigen-binding when antibody-tagged (Taylor et al. 2005).
- Archaeosomes, which are liposomes made from Archaeobacteria, are even more thermostable and more resistant to oxidation, low pH, bile salts, chemical and enzymatic hydrolysis compared with normal liposomes and therefore considered ideal candidates to protect antioxidants during food processing.
- Nanocochleates have a cigar-shaped multilayered structure consisting of a continuous, solid, lipid layer sheet rolled up in a spiral fashion with little or no internal aqueous space. Nanocochleates have been used to deliver proteins, peptides and DNA for vaccine and gene therapy applications. They are resistant to degradation in the gastrointestinal tract, which makes them ideal candidates for oral delivery.

Because of the natural composition of liposomes it may appear that there are no safety concerns if liposomes are used in food industry. However, as was pointed out by Mozafari et al. (Mozafari et al. 2006), the utilization of above mentioned lipid-based carriers in the area of food is determined by their preparation procedure, which may not involve non-food grade solvents and detergents.

Polymer-based nanoencapsulation:
Nanoencapsulates based on polymers can be obtained by the polymerization of more than one type of monomer, typically one hydrophobic and one hydrophilic, so that the resulting molecule is composed of regions that have opposite affinities for an aqueous solvent. Numerous copolymers have been synthesized to date, generally composed of a biocompatible, biodegradable hydrophobic polymer block covalently bounded to a biocompatible hydrophilic polymer block, leading to the formation of micelles, nanospheres, polymersomes and nanocapsules (Kabanov 2006; Letchford and Burt 2007). In figure 3.2 the delivery systems formed by amphiphilic copolymers are shown.
- Micelles are characterized by a core-shell architecture in which the inner core is composed of the hydrophobic regions of the amphiphilic molecules creating a cargo space for the lipophilic drug or compound.
- Nanospheres can be defined as a solid colloidal particle in which drugs are dissolved, entrapped, encapsulated, chemically bound or adsorbed to the polymer matrix. However, the central core can be become more or less solid-like depending on the copolymer composition, making if difficult to have a clear distinction between micelles and nanospheres.
- Nanocapsules and polymersomes are colloidal-sized, vesicular systems in which the drug is confined within a cavity surrounded by a polymer membrane or coating. If the core is an oily liquid and the surrounding polymer a single layer the vesicle is referred as a nanocapsule; these system have found utility in delivery of hydrophobic compound. If the core of the vesicle is an aqueous phase and the surrounding coating is a polymer bilayer, the particle is referred to as a polymersome. These vesicles are analogous to liposomes and find utility in delivery of encapsulation of water-soluble compound, but they differ from liposomes in that the external bilayer is composed of amphiphilic copolymers. Variation in composition, molecular geometry and relative monomer lengths results in various physicochemical properties and morphologies of the resulting nanoencapsulates (Letchford and Burt 2007).

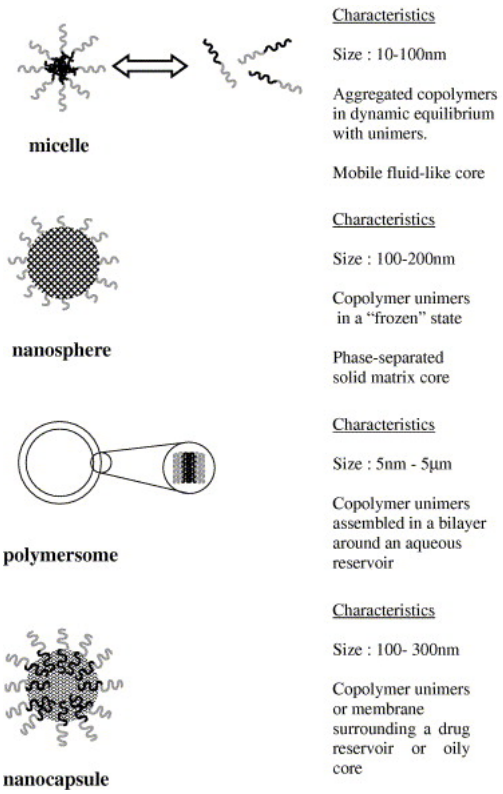


Figure 3.2: Nanoparticulate drug delivery systems formed by amphiphilic copolymers and their general characteristics. Taken from (Letchford and Burt 2007).

In food industry food-grade polymers have to be utilized. For instance, polyethylene glycol (PEG) is generally used as a hydrophilic polymer block in the formation of nanoencapsulates (Letchford and Burt, 2007) and generally recognized as safe. Moreover, protein-based nanoencapsulates are particularly interesting because they are relatively easy to prepare and can form complexes with polysaccharides, lipids or other biopolymers and a wide variety of nutrients can be incorporated (Chen et al. 2006b). Natural polymers used for the formulation of nano delivery systems are albumin (protein), gelatin (protein), alginate (saccharide), collagen (protein), chitosan (saccharide) (des Rieux et al. 2006) and the milk protein alpha-lactalbumin (Graveland-Bikker and de Kruif 2006).

4 Scientific data for risk assessment of nanoparticles in food

4.1 Physicochemical characterization of nanoparticles, stability in the food matrix and availability of analytical tools

Engineered nanoparticles (NPs) encompass many forms. They can be made bottom up, through assembling molecules into NPs, or derived top down by down sizing conventional substances. A complete and accurate characterization of NPs (Oberdorster et al. 2005a; Powers et al. 2006) is an essential part of both understanding the possible benefits as well as the potential toxicity of NPs in biological systems (Royal Society, 2004). Whereas the characterization of chemicals is usually relatively straightforward (e.g. composition, purity), characterization of NPs in biological matrices is more complex (Powers et al. 2007). The novel properties of NPs are primarily associated with their small size.
There is a commonly used definition which states:

> *"Engineered NPs materials are designed and produced to have structural features with at least one dimension of 100 nanometers or less (Oberdorster et al. 2005a)"*

Nanotechnology involves the manufacture, processing and application of materials that are in the size range of 100 nanometers or less. The size limits have once been chosen on an the basis of physico-chemically relevant properties, but have however no toxicological basis at all.
In international fora like Scientific Committee on Emerging and Newly-Identified Health Risks (SCENIHR) and the International Organization for Standardization (ISO) discussions on definition are high on the agendas. International consensus on a definition will have its impact on regulatory frameworks. At this moment discussions seem to lead in the direction of defining the upper size limit of a NP is as *approximately* 100 nm, which is not strict enough for application in regulatory frameworks. It is however important to realize that the size may not be the only relevant descriptor of a dose of NPs e.g to explain these novel properties of NPs. Other characteristics that need to be considered are (Muller et al. 2001; Oberdorster et al. 2005a; Powers et al. 2006):

- size and size distribution,
- agglomeration state
- shape and other morphological features (e.g. crystallinity, porosity, and surface roughness),
- chemical composition,
- solubility,
- total surface area,
- surface chemistry
- surface charge (zeta potential)

Toxicity testing of NPs requires that in the end dose-respons relationships can be described, either for in vivo or in vitro tests. As already mentioned above, the conventional approach on the basis of mass in describing the dose of a compound will not hold (SCENIHR Opinion, 2007). However up till now it has not been possible to establish a single alternative dose-describing parameter that describes the dose and the observed dose response relations in toxicological tests well. This leads to a general recommendation that NPs used for (toxicological) studies should be characterized as completely as possible (Oberdorster et al. 2005a) (Powers et al. 2006) (Thomas and Sayre 2005).

This leads to a general recommendation that NPs used for (toxicological) studies should be characterized as completely as possible (Oberdorster et al. 2005a; Powers et al. 2006; Thomas and Sayre 2005). For a comparison of studies and determining the accuracy of the methods used in different laboratories, it is important to assess the precision of analytical (screening) methods, lab-to-lab variation and effects of method development (Kroes et al. 2002). This is not only important for the safety evaluation but it is also important to be able to model NP properties *in silico* in the future (e.g. QSAR) (Powers et al. 2007).
It might however not be possible to fully characterize the NPs. In an attempt to give some guidance on prioritization of characterization of NPs Oberdörster and coworkers (Oberdorster et al. 2005a) proposed three criteria: "the context within which a material is being evaluated, the importance of measuring a specific parameter within that context, and the feasibility of measuring the parameter within a specific context". Part of this information is currently presented in the relevant dossier parts, but it is clear that more information should be requested.
Physicochemical characterization of NPs is important to be able to explain observed effects in test systems. Characterization of NP as-produced will provide useful baseline data of the NPs. But for toxicity studies and (consumer) exposure assessment, it is crucial that this characterization is performed in the matrix containing the NPs as administered to test systems (or consumer) (Oberdorster et al. 2005a). It is clear that the functionalities of the NPs (e.g. particle size, size distribution, potential agglomeration and surface charge), can change in different biological matrices (Powers et al. 2006), depending on compounds that are present in the matrix and thermodynamic conditions (Borm and Kreyling 2004). In addition NP interactions with the matrix can change as a result of dilution (Oberdorster et al. 2005a). In practice this means that the appearance of a NP can be expected to change following sample processing (e.g. freeze - thaw cycles, heating, dilution). A special case is NP incorporation in packaging materials. The rate of migration of NPs from packaging materials is not known a priori. The migration of heavy metals from biodegradable starch/clay nanocomposite films to be used as packaging materials, was shown to be minimal (Avella et al. 2005). It is not clear however whether the results from this study are applicable in general. Furthermore the adequacy of current migration tests and cut off migration limits need to be considered. And for a thorough safety evaluation the NPs will also have to be characterized after migration into the food matrix.

At present there is a vast array of analytical techniques to characterize NPs. The mean size and width of distribution (polydispersity index) of nanosuspensions is typically determined by photon correlation spectroscopy (PCS). The measurement range of this technique is limited to approximately 3 nm-3um. Therefore, additional laser diffractometry (LD) with a measuring range of approximately 0.05 –80 um is used to detect any presence of particles in the micrometer range or aggregates of NPs (Muller et al. 2001). Analysis by the Coulter counter technique gives absolute data (i.e. absolute number of particles per volume unit for the different size classes).
Other methods to determine the size distribution of NPs are light scattering, differential mobility analysis, time of flight mass spectroscopy (TOF-MS), microscopy and surface area measurements (Powers et al. 2006).
Particle charge is generally measured by electrophoresis and typically expressed as electrophoretic mobility [(um/S)/(V/cm)] or converted to the zeta potential (mV) (Muller et al. 2001; Powers et al. 2006).

The crystalline structure of a nanosuspension can be assessed by differential scanning calorimetry (DSC) (Muller et al. 2001) polarized optical microscopy and scanning electron microscopy (Avella et al. 2005).
For determining hydrophilicity/ hydrophobicity (important characteristic affecting the in vivo organ distribution (after i.v. injection) can be determined with hydrophobic interaction chromatography (HIC) (Luck et al. 1997).
NPs are often coated with proteins in order to modify the surface characteristics (e.g. to increase adhesion to the gut wall, (Muller et al. 2001)).Qualitative and quantitative composition of protein absorption patterns can be analyzed by 2-D PAGE. Molecular composition and structure of the surface of NPs can be determined by electron spectroscopy for chemical analysis (ESCA), X-ray photoelectron spectroscopy (XPS) and secondary ion mass spectroscopy (SIMS), or a technique called matrix assisted laser desorption ionization microscopy/mycroscopy (Powers et al. 2006).
For inert NPs the surface area is important, because interaction between NPs and the biological matrix typically take place at the particle's surface. Surface area of these type of NPs is normally measured through gas adsorption using the Braunauer, Emmett and Teller (BET) method or other theoretical approaches. These measurements are difficult in biological matrices (Powers et al. 2006).
In general, all these techniques require a well equipped laboratory and were developed for characterization of NPs during the production phase (in simple matrices), water (buffer) or in the air. Literature on validated methods for the characterization of NPs in biological or food matrices is scarce.

4.1.1 In vitro testing

The development of routine analytical techniques for the characterization of NPs might be very difficult to achieve. A completely different approach can be the determination of the presence of NPs by means of effect screening in analogy with screening approaches used for complex mixtures of chemicals and products derived from genetically modified organisms in food. In this approach the presence of NPs can be "detected" focusing on biomarkers of exposure or effect of developed assay systems. The *in vitro* assays could then be used in a first tier of detection of NPs in food. However, suspected samples whould have to be further characterized by means of analytical techniques.

4.1.2 Summary / interpretation physicochemical characterization

Complete information on relevant physicochemical properties of engineered NPs is considered to be essential for proper risk assessment. This information is required for development and confirmation of theoretical approaches of dose metrics. First then it will be possible to evaluate and compare the results of toxicity studies in a proper way.
The size limit in the present definition of NPs is still arbitrary due to lack of knowledge on the relationship between particle size, kinetics and effects. It is thus not known whether the size-range of interest is exactly at 100 nm or below or above. Otherwise, it will be relevant to explore the legal feasibility of avoiding arbitrary size limits, in order to handle the consequences of scientific uncertainties in a more pragmatic way. Such knowledge is not easily derived. The definition should therefore first be treated in a pragmatic way.
It has become clear that the size will not be the only descriptor of a dose e.g not be the only factor to explain these novel properties. Total surface area or number of particles are also considered to be relevant descriptors. In practice NPs in food will cover a certain range of sizes (distribution) and the particles will have a variety of shapes. These two issues already imply that doses cannot be described on a weight or volume basis, but it is also to simple to assume that a one dimensional parameter like surface area can be a good substitute.Probably, multifactorial units, taking into account all relevant

parameters will need to be developed. .As stated above, it will be challenging to combine these parameters to one relevant dose descriptor.
Dose response relations in toxicity tests will have to be analyzed case by case using different dose-describing parameters. Furthermore, since the functionalities of the NPs (e.g. particle size, size distribution, potential agglomeration and surface charge), can change in different biological matrices, or as a result of dilution, it is crucial for toxicity studies and (consumer) exposure assessment that the physicochemical characterization is performed in the matrix containing the NPs as administered to test systems (or consumers) (Oberdorster et al. 2005a).
Regarding the present knowledge on toxicity of NPs it is important for risk assessors to have access to a clear description of the analytical methods that were used to determine the physicochemical properties of the respective NP, to have access to the (raw) experimental data and a sound description of the statistical procedure used to analyze the data. Only then a reliable assessment of the NPs can be performed and only then the results of the safety assessment can be used to model NP properties in silico (Powers et al. 2007), or compare results with those of non-nano counterparts.
Future research should focus on methods that are able of in situ detection and characterization of NPs, and that are preferably relatively easily performed with apparatuses that are currently present at laboratories suited for detection of chemicals in food. Ideally, isolation and characterization methods should be developed, suitable for routine and low-cost analysis.
The use of in vitro effect assays might be considered as the first tier for gaining insight in potential presence and toxicity of NPs in certain products. In this approach the presence of NPs can be "detected" focusing on biomarkers of exposure or effect of developed test systems. Their application might be less time-consuming and expensive on one hand, but on the other hand the results of these types of assays are only to be used in a qualitative or semi-quantitative way.

Several international working groups, like ISO and the EU Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) are considering definitions of nanotechnologies and NPs that both adequately describe the novel nature of NPs and are applicable in regulatory frameworks. A generally accepted definition of NPs is essential for all stakeholders, i.e. regulators, producers and researchers.
Knowledge on dose-describing parameters will be important imput for these discussions. A proper dose metrics will help researchers to compare study results and will help regulators to formulate limit values. It will also enable risk assessors to compare and combine exposure and hazard information and conclude on the likelihood of health risks.

4.2 Toxicokinetics of nanoparticles

The potential toxicity of NPs is determined by their toxicokinetic (Figure 4.1) and toxicodynamic behaviour, i.e. the absorption (uptake), metabolism (biotransformation), distribution (allocation) and excretion (elimination) characteristics in combination with the interaction of the substance with target sites and the subsequent reactions leading to adverse effects. Since NPs show remarkable structural diversity (such as tubes, dots, wires, fibres and capsules) with each structure exhibiting its own individual characteristics (Ballou et al. 2004; Roszek et al. 2005; Singh et al. 2006) it is reasonable to assume that these deviations in properties may lead to different toxicokinetic and toxicodynamic effects. When compared to their macrosized counterparts, all nanoscale materials have physical, chemical, optical, electrical, catalytical and mechanical properties that may differ fundamentally leading to a

different distribution pattern (Preining 1998). Furthermore, the relative large surface area compared to the volume of NPs can make them more reactive than larger particles, which may increase the potential toxicity. Although scientific knowledge on the potential toxicity of NPs is limited, several studies suggest that NPs may have a different toxicity profile when compared to their larger counterparts (Donaldson et al. 2001; Oberdorster et al. 2005a).
Analysis of the available literature of toxicokinetics of NPs of various nature and type (in drugs, food, non-food products) demonstrated that no general conclusions on Absorption, Distribution, Metabolism and Excretion (ADME) for NPs can be drawn. As stated before, study results from various studies can hardly be compared due to a lack of proper dose metrics and characterization of the NPs applied in the studies. No coherent set of studies regarding *certain* types of NPs appeared even to be available.

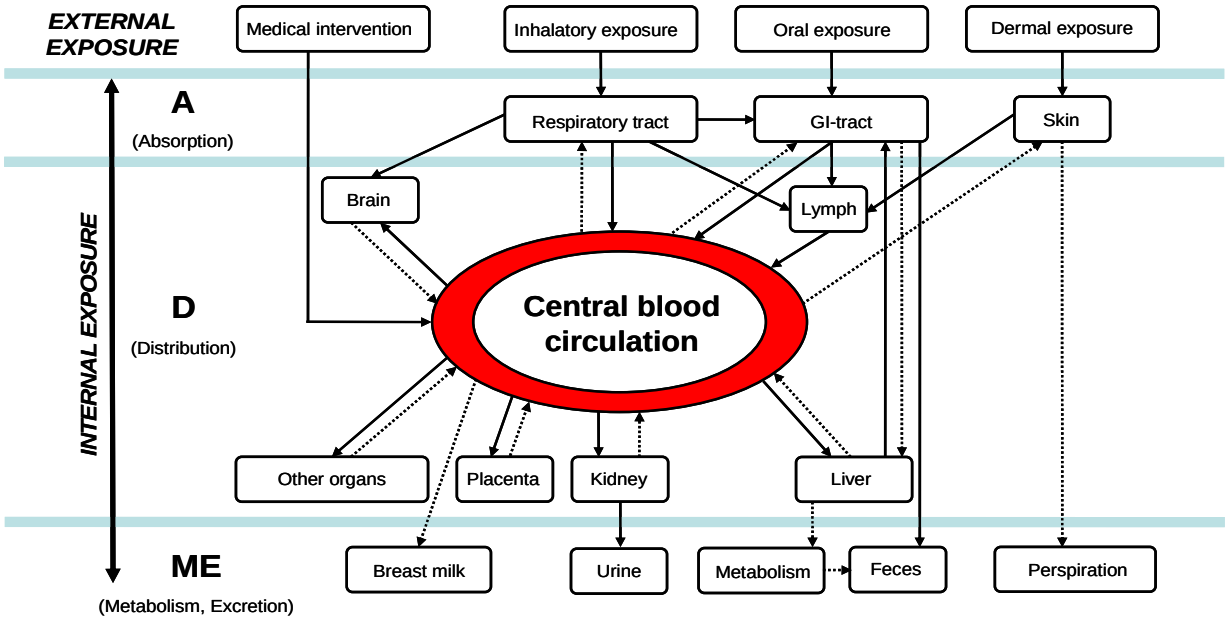


Figure 4.1: The ADME processes (absorption, distribution, metabolism and excretion) of NPs in the human body. The internal exposure is the part of the external dose that reaches the systemic circulation. The black lines represent confirmed routes for NPs; the dashed lines represent hypothetical routes. The transport rates and retention times for the indicated processes are largely unknown. (Other organs are: e.g. spleen, heart, reproductive organs. Modified from (Oberdorster et al. 2005b).

4.2.1 Absorption

Following oral uptake, NPs as well as nanoencapsulates have to pass the gastrointestinal epithelium before they enter the liver and subsequently the systemic circulation. This epithelium primarily consists of enterocytes, representing the majority of the cells, goblet cells and M-cells. The latter are localized in the Peyer's Patches. In theory, particles can cross the epithelium by two main routes: paracellular (between the cells, through the tight junctions) and transcellular (through the cells). The rate of particle uptake via one of abovementioned routes is depending on the properties of the nanoparticle or nanoencapsulate (e.g. size, hydrophobicity, surface charge or surface coating).
The first uptake route of nanoparticals or nano-encapsulates is the paracellular route (Figure 4.2). The total area of the paracellular route has been reported to range from 0.01% (Pappenheimer and Reiss, 1987 in (Salamat-Miller and Johnston)) to 0.1% (Nellans, 1991 in (Salamat-Miller and Johnston), corresponding to a surface area of 200 to 2000 cm^2 (Salamat-Miller and Johnston 2005). Cells of the

gastrointestinal epithelium are tightly connected to each other by means of tight junctions. However there is body of evidence that is indicating that the intestinal epithelium is permeable to large proteins and polypetides (Salamat-Miller and Johnston 2005). It has become clear that the permeability of the tight junctions can be modulated; some polymers can act as expanders of the tight junctions thereby introducing a port of entry for many particles as well as an entrance for toxins, bacteria and immunogens (Salamat-Miller and Johnston 2005). Moreover, it is not fully known whether these effects are reversible and hence if the opening of the cellular barrier is transient (Salamat-Miller and Johnston 2005).

The other uptake route is the transcellular route and describes the process by which particles are taken up at the apical side of the intestinal epithilium by endocytocis, transported through the entrocytes and subsequently released at the basolateral side of the intestinal epithilium. It is generally acknowledged that nanoparticle (50nm – 20 μm) translocation primarily occurs in the Peyer's Patches via the M-cells and to a lesser extent via the enterocytes. There is however evidence that the particle uptake and transportation via enterocytes cannot be neglected (Aprahamian et al. 1987; Carr et al. 1996; des Rieux et al. 2006; Florence 2005; Hillery et al. 1994; Hoet et al. 2004; Jani et al. 1990)(Figure 4.2), coating of the nanoparticle with e.g. lectins will stimulate enterocytes to endocytose the NP (Russell-Jones et al. 1999).

Initially it was assumed that the Peyer's Patches did not discriminate strongly in the type and size of the absorbed particles. Later it has been shown that modified characteristics, such as particle size, the surface charge of particles, attachment of ligands or coating with surfactants, influences the uptake of particles by the gastrointestinal tract (Hoet et al. 2004). Small polystyrene NPs appeared to be more easily absorbed compared to larger particles (Desai et al. 1996; Jani et al. 1990). In addition, charged particles exhibited poor oral bioavailability through electrostatic repulsion and mucus entrapment compared to non-ionic hydrophobic particles(Florence 1997; Florence 2005; Hoet et al. 2004; Hussain et al. 2001).

Specific for the M-cells is the transepithelial vesicular transport with little or none of the endocytosed material directed to lysosomes. It is not clear to what extent endocytosed materials are degraded or processed during transepithelial transport (Kraehenbuhl and Neutra 2000). Endocytosed intact encapsulates or NPs might thus enter both the blood and lymphoid circulation (Gabor et al. 2004)A special aspect of nanoencapsulates is their interaction with membranes of epithelial cells. For instance (synthetic) polymers have been shown to interact with charged groups of membrane proteins. That may affect cell signaling processes involved in inflammation, differentiation, proliferation and apoptosis, interfering with normal cell function (Kabanov 2006). Moreover, synthetic polycationic polymers caused nanoscale hole formation in lipid bilayers allowing the diffusion of molecules in and out. At higher concentrations the polymers even caused substantial membrane damage resulting in cell death (Hong et al. 2006).

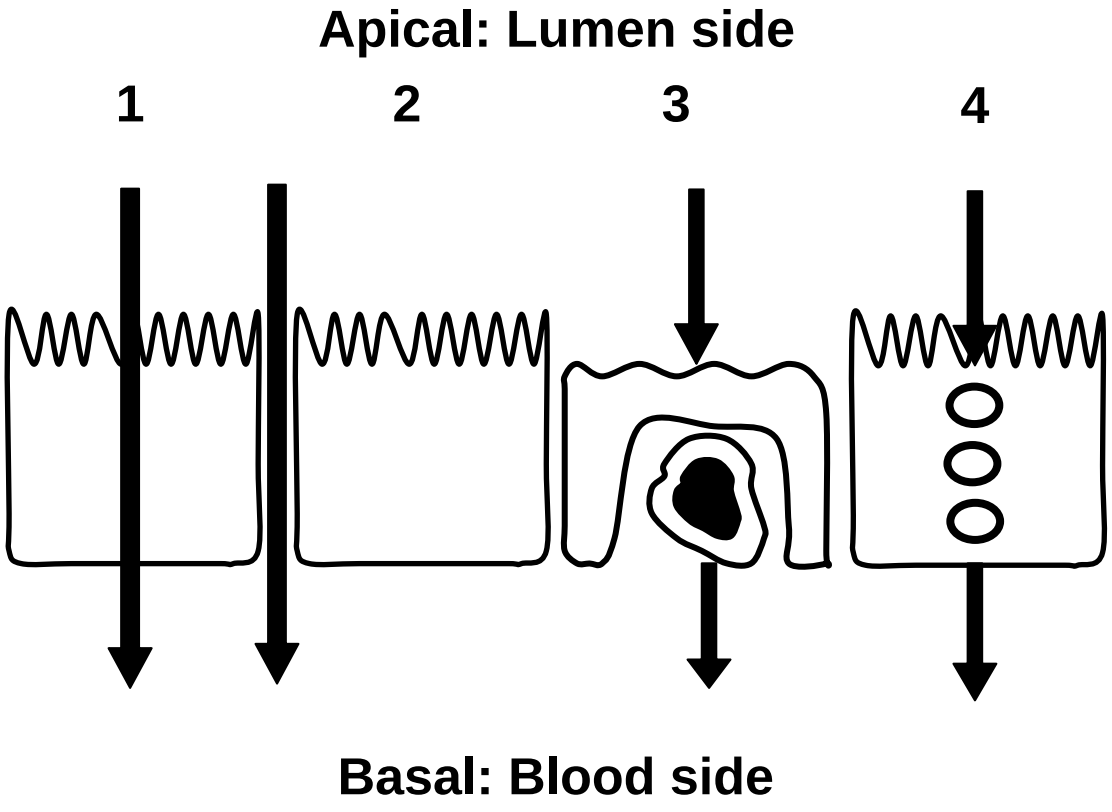


Figure 4.2: Transport mechanisms for NPs (Modified from (des Rieux et al. 2006)). 1 = Passive diffusion, 2 = Paracellular transport, 3 = Trancytosis by M-Cells, and 4 = Transcytosis by enterocytes.

4.2.2 Distribution

After the absorption process of NPs by the various ports of entry, the systemic circulation can distribute them towards all organs and tissues in the body (Figure 4.1). Several studies have shown distribution of of NPs (including C_{60} fullerenes, ^{99m}Technetium-labeled carbon NP, ^{192}Iridium NP, ^{13}Carbon NP and colloidal gold NP) to a variety of target organs including liver, spleen, kidneys, bone marrow, lungs and brain (Borm et al. 2006; Hillyer and Albrecht 2001; Ji et al. 2006; Nemmar et al. 2002; Oberdorster and Utell 2002).

4.2.2.1 Distribution following oral exposure

Oral uptake (gavage) of polystyrene spheres of different sizes (50 nm to 3 micron) in female Sprague Dawley rats resulted in a size dependent systemic distribution of the NPs. About 7% (50 nm) and 4% (100 nm), was found in the liver, spleen, blood and bone marrow. Particles larger than 100 nm did not reach the bone marrow and those larger than 300 nm were absent from blood. No particles were detected in heart or lung tissue (Hoet et al. 2004; Jani et al. 1990).

Hillyer and Albrecht showed that after oral administration of metallic colloidal gold NPs of decreasing size (58, 28, 10 and 4 nm) to mice an increased distribution to other organs was observed. The smallest particle (4 nm) administered orally resulted in an increased presence of gold particles in kidney, liver, spleen, lungs and even the brain. The biggest particle (58 nm) tested was detected almost solely inside the gastrointestinal tract (Hillyer and Albrecht 2001). As to be expected, this suggests that both the absorption and distribution of this type of NPs is size dependent (Hillyer and Albrecht 2001; Jani et al. 1990).

4.2.2.2 Systemic circulation

When NPs or nano-encapsulates reach the systemic circulation, the particles can, potentially, interact with plasma-proteins, coagulation factors, platelets and red and white blood cells. This interaction is depended on the surface chemistry of the particle as is shown by Nemmar et al. (Nemmar et al. 2002). In this study, carboxylate-polysterene, amine-polysterene and unmodified polystyrene (60nm) particles revealed distinct tendency to induce thrombis formation after intravenous and intratracheal administration in hamsters. In addition, the binding of NPs to plasma components may have a substantial effect on their distribution and excretion. For instance, the hydrophobic surfaces of nanospheres are highly susceptible to opsonization and clearance by the reticuloendothelial system, resulting in sequestering of the particles within organs such as the liver and spleen (Letchford and Burt 2007).

Several different NPs (gold and titanium oxide) have been identified inside human red blood cells (Rothen-Rutishauser et al. 2006) Interestingly, this cellular uptake of NPs did not involve endocytosis or phagocytosis (Geiser et al. 2005) since erythrocytes do not have phagocytotic receptors (Rothen-Rutishauser et al. 2006). This suggests that NPs are able to cross the cell membrane by processes other than phagocytosis and endocytosis. Diffusion, transmembrane channels, adhesive interactions or other, undefined, transmembrane processes might play an important role in this cellular uptake. The intracellular NPs of this type appeared to be not membrane bound and might have direct access to the intracellular proteins, organelles and DNA of the cell, which might imply enhanced toxic potential (Geiser et al. 2005).

Depending on the physico-chemical properties of the nanoencapsulates, they can be internalized and release their content in the cytoplasma or remain intact for cellular uptake. Poly (D,L-lactide-coglycolide) NPs showed efficient endocytosis (Panyam and Labhasetwar 2003). In addition, nanoencapsulates in lipsomes can also enter the cell by fusion of the liposome with the cell membrane (Ulrich 2002). It needs to be further explored what the effect of encapsulates including compounds is on membrane integrity and cell function.

4.2.2.3 Trans-placental distribution

Data addressing the distribution of NPs to the reproductive cells is, as yet, unavailable. In addition, no clear data is available identifying the distribution of NPs in the fetus. An in vivo study in which pregnant mice were intraperitoneally injected with soluble C60 fullerenes indicated transfer into the embryo via trans-placental passage of the maternal blood flow (Tsuchiya et al. 1996). However, a direct passage from the peritoneal cavity into the uterus could not be excluded.. Nevertheless, reproductive toxicity, including excretion via breast milk, needs serious attention as this is an important issue for risk assessment.

4.2.2.4 Distribution to the brain

NPs may enter the brain by two different mechanisms. The first process involves a transsynaptic transport of NPs through the olfactory epithelium (Elder et al. 2006; Oberdorster et al. 2005a). The second pathway involves a direct passage of the particle through the Blood Brain Barrier (BBB). This physiological barrier controls the passage of substances from the blood into the central nervous system. The permeability of this barrier is highly restricted to molecules which are either lipophillic, actively transported or are small soluble molecules (< 500 Da). This barrier may therefore represent a strict defense mechanism from blood borne particle exposure that limits the distribution of NPs to the brain. However, evidence exists that distribution to the brain might occur for some NPs, since low concentrations of gold were found in the brain after oral administration of gold (4-58 nm) NPs (Hillyer

and Albrecht 2001). It is suggested that the surface area of NPs is important in blood-brain integrity and need consideration as to their role in brain toxicity and brain distribution (Borm et al. 2006). Moreover, specific coating of NPs and nanoencapsulates are intended to create the possibility for the development of nanosized drug delivery systems to enable specific blood brain barrier crossing and distribution to the brain (Silva 2007).

4.2.3 Metabolism

Once NPs are absorbed by the gastrointestinal tract, these particles will be transported directly to the liver via the portal vein. The liver is able to actively remove compounds from blood. At this moment there is no evidence that this "first pass effect" also plays a role for any type of NPs. In general, this first pass effect can be considered as an elimination mechanism of the parent compound. For that reason oral bioavailability (fraction of the dose administered which reaches the systemic circulation) often is lower than the fraction absorbed from the intestinal lumen. A study in rats showed a rapid elimination of polystyrene NP (50 nm) following single intravenous administration. These particles distributed predominantly in the hepatocytes and Kupffer cells in the liver. Twenty four hours after intravenous administration. 4% of the dose was excreted via the biliary route (Ogawara et al. 1999; Ogawara K. 1999).

Studies on metabolism of NPs have not been reported thus far. It is unlikely that inert NPs such as gold and silver particles, fullerenes and carbon nanotubes, can be metabolized effectively by enzymes in the body. However, it could be hypothesized that specific NPs with functionalized groups can be metabolized. For instance, the protein cap of a functionalized quantum dot could be cleaved by proteases ((Hardman 2006). Then the metallic core of quantum dots (and other metal oxides) could be bound by metallothionein and excreted. These enzymes, present in liver and kidney, can bind metal and restore the cellular metal homeostasis (Coyle et al. 2002). In addition, NP drug delivery systems consisting of liposomes are able to fuse with cell membranes. The intracellularly released drug could be metabolised according to the normal metabolism pathway described for the conventionally formulated drugs.

Borm (Borm et al. 2006) posed several mechanisms for elimination of NPs. Free particles are likely to be removed from the circulation via phagocytic cells in the reticulendothelial system and hence will accumulate in organs such as liver and spleen. The consequence of nanoparticle uptake by macrophages is not yet known, there is evidence that it may stimulate the production of proinflammatory cytokines as was shown in alveolar macrophages (Brown et al. 2002) and then affect the functioning of e.g. the liver.

4.2.4 Excretion

An absorbed NP in the systemic circulation can be excreted by various routes. They can be distributed to the liver, taken up by hepatocytes and excreted in the bile to the gastrointestinal tract. Another possible elimination route for NPs could be renal clearance. Indeed, this latter route has been found to clear fullerenes and single walled carbon nanotubes (SWCNT) from the body (Rajagopalan et al. 1996; Singh et al. 2006). The plasma half-lives following intravenous injection in rats have been determined for several NPs (C60; C82; SWCNT) (Cagle et al. 1999; Rajagopalan et al. 1996; Singh et al. 2006). Interestingly, the study with C82 fullerenes suggested a prolonged circulation time compared to the C60 Since, the fullerenes used in these studies have different functionalized groups it was suggested that not only size but also the chemical properties of NPs influence its excretion (Sayes et al. 2004)

A size dependent excretion has also been suggested via bile. After intravenous administration in rats, polystyrene NPs were taken up by the liver and subsequently excreted in the bile. These particles larger (50 nm) were phagocytosed by Kupffercells partly and partly taken up by the hepatocytes, whereas

polystyrene microparticles (500 nm) were taken up predominantly by the non-parenchymal cells (Kupffercells and endothelial cells) (Ogawara et al. 1999).

4.2.5 Summary /interpretation of ADME

Taken together, the toxicokinetic properties of NPs for the Absorption, Distribution, Metabolism and Excretion (ADME) processes seem to depend strongly on the size of the particles as well as on the charge, additional functionalized groups and other novel physico-chemical properties. Thus no general conclusions on ADME processes for NPs can be drawn. This was to be expected because NPs cannot be considered as one sort of compound. Exposure within tissues and organs might for that reason become higher than following an equivalent dose of the conventional chemicals form (Preining 1998). As stated before, study results from various studies can hardly be compared due to a lack of proper dose metrics and characterization of the NPs applied in the studies, which hampers to establish a relationship between physicochemical properties and kinetic behavior (as is more or less the case for conventional chemicals). No coherent set of studies regarding *certain* types of NPs appeared even to be available.
Animal studies have indicated nanoparticle absorption through the intestine and subsequent distribution to several target organs (including the liver, spleen, kidneys, bone marrow, lungs and brain). Due to the potential impact on toxicological effects, special attention needs to be paid to observations that some NPs are capable of crossing biological barriers such as the blood-brain barrier and the placenta. Also on cellular level, barriers such as cell membranes do not constitute obstacles for some tested NPs. The excretion of NPs (metabolic and elimination processes) remain poorly understood.
For nanoencapsulates (including its contents) the distribution and elimination is thought to be influenced by the interaction with the reticuloendothelial system, which is composed of phagocytic cells in the liver, spleen and lymph nodes. These nanoencapsulates may end up in liver and/or spleen, which may be different from the target organ for the conventional form of the (non encapsulated) compound.
Moreover, they may end up in different cell organelles and/or persist there for a longer period. Also, compounds that are normally prevented from entering an organ by a specific barrier may now enter this organ when presented as a nanoencapsulate. This opens up the possibility of specific drug targeting to organs and cells which would otherwise not be treated effectively.
Since the effects of particle size, charge, coating and other phycisochemical properties on the kinetic behavior of NPs and nanoencapsulates are unknown in great detail, future research should focus on determination of the specific kinetic parameters and processes such as the half-live and elimination (metabolism and excretion) routes in several species. Also the distribution of the particles to target organs and the transplacental and blood-brain barrier passage should be studied in more detail to ensure safe application of nanoparticle containing consumer products. As validated toxicokinetic data of more NPs will become available, more reliable extrapolation to other exposure scenarios than applied in the study will become possible in the (near) future.

4.3 Toxicodynamics of nanoparticles

Most information on the potential toxicity of NPs is available from inhalation toxicity studies in animals and humans. For the use of NPs in food, however, oral exposure is the most important route of exposure. The following paragraphs give an overview of the available information on the toxicity of NPs after oral exposure and if not available, also for other exposure routes. It should be kept in mind that results are often obtained for only one type and size of NPs. Extrapolation from one type of NPs to another, or from one size to another is on the basis of present knowledge still impossible. At this moment there is too few data to draw conclusions for which type of effects are to be expected for which type of NPs..

4.3.1 Acute and subchronic toxicity

Acute, subacute and subchronic toxicity after oral exposure have been investigated in rodents for several different NPs (see Table 4.1 and Annex 2).

Table 4.1: Summary table of available oral toxicity studies of NPs

Study	Species	Nanoparticle	Results	Reference
Acute and subacute toxicity	Rats	C60 polyalkyl-sulfonate	No mortality after doses up to 2500 mg/kg	(Chen et al. 2006c)
Acute toxicity	Mice	Zinc (58 + 16 NP)	Increased mortality, renal damage and anemia after 5 mg/kg compared to microscale zinc (1.08 + 0.25 μm)	(Wang et al. 2006)
Acute toxicity	Mice	Copper (23.5 NP)	Increased mortality, renal, liver and spleen damage compared to micro-particles	(Chen et al. 2006c)
Acute toxicity	Mice	Titanium dioxide (25 and 80 NP)	No increased mortality, but increased liver and kidney damage compared to fine particles (155 NP)	(Wang et al. 2007)
Acute toxicity	mice	Selenium	Lower acute toxicity compared to selenite (LD50 113 compared to 15.7 mg/kg bw)	(Zhang et al. 2005)
Short term toxicity	Mice	Selenium (20~60 NP)	Less oxidative stress, liver injury and growth retardation compared to selenite	(Zhang et al. 2005)
Subchronic toxicity	Rats	Selenium (20~60 NP)	Less toxic than selenite and high-selenium protein	(Jia et al. 2005)
Acute and subchronic toxicity	mice	cationic PAMAM dendrimers	Three animals died after single administration of 45 mg/kg and liver toxicity was observed after multiple dosing.	(Duncan and Izzo 2005)
Acute toxicity	mice	pure and N-doped carbon multi-walled nanotubes	No signs of distress or tissue changes.	(Carrero-Sanchez et al. 2006)
Acute toxicity	mice	nano-magnetic ferrrofluid (magnetic particles about 19.9 NP)	Low toxicity (LD50: >2104.8 mg/kg, maximum non-effect dose (ED0): 320 mg/mg)	(Xia 2005)

The results of the available oral toxicity studies indicate that, depending on the particle size, coating and chemical composition of the NPs, acute toxicity at high doses may occur. The main target organs appear to be the liver and kidney, but adverse effects in the blood, heart and spleen have also been observed. No information on the toxicity after chronic or acute low dose oral exposure is available.

4.3.2 Toxicity of cardiovascular system

Cardiovascular effects have been observed following inhalation exposure. These effects may be caused directly by NPs entering the blood or indirectly by inflammatory reactions in the lungs (Borm et al. 2006; Tran et al. 2005). In case of a direct mechanism of action, particles absorbed by the lung

endothelium and present in the central blood circulation might have direct effects on the endothelium, plaques and thrombogenic mechanisms (Tran et al. 2005). Although cardiovascular effects have not been described yet after oral exposure, it might be considered a plausible effect of systemically available particles.
In addition to the described effects on lung, liver, brain and cardiovasculair system, NPs may reach the heart and the bone marrow where they may affect the cardiomyocytes and other cells of the heart directly (Tran et al. 2005) or cause a variety of effects on immunity and haemopoesis respectively (Oberdorster et al. 2005a; Tran et al. 2005).

4.3.3 Toxicity of reticulo-endothelial system

As stated before the liver is important to actively remove compounds from the blood of the portal vein: the “first pass effect”. In the gastrointestinal tract phagocytotic cells are in place to also actively remove particles. This reticulo-endothelial system consists of cells (macrophages, monocytes, and specialised endothelial cells) that have the ability to phagocytose cellular debris, aged cells, pathogens and foreign substances including NPs from the blood stream. In this way potential pathogens that enter the body from the gastrointestinal tract microflora are removed and neutralized. The consequences of nanoparticle uptake are unknown, however, in vitro studies demonstrated the generation of proinflammatory cytokines via reactive oxygen species and calcium signaling. Oxidative stress is known to inhibit hepatocyte function and bile formation, while pro-inflammatory cytokines are also associated with the pathology of liver disease. Hence, the impact of NPs on the liver and reticulo-endothelial system needs to be investigated (Borm et al. 2006).

4.3.4 Neurotoxicity

The Blood Brain Barrier (BBB) forms a physiological barrier that limits the distribution of NPs from the blood to the brain. NPs can, however, to some extent enter the brain and a number of pathologies, including hypertension and allergic encephalomyelitis, however have been associated with increased permeability of the BBB to NPs in experimental setups. Conversely, the nanoparticle surface charge has been shown to alter blood-brain integrity (Borm et al. 2006) and needs consideration as to its role in brain toxicity and brain distribution (Borm et al. 2006). Moreover, specific coating of nanoencapsulates may also enable the encapsulates to cross the BBB and enter the brain (Silva 2007). In addition, a number of pathologies, including hypertension and allergic encephalomyelitis, have been associated with increased ability of NPs to penetrate the BBB in experimental setups.
The potential impact of NPs on human neuronal tissue is as yet not investigated in detail. However, NPs have been shown to induce the production of reactive oxygen species and oxidative stress has been implicated in the pathogenesis of neurodegenerative diseases such as Parkinson's and Alzheimer's disease. It is conceivable that the long term effects might include a decrease in cognitive function. Increased markers of inflammation and AB42-accumulation in frontal cortex and hippocampus in association with the presence of NPs have been found. Additionally, inhalation exposure of BALB/c mice to particulate matter showed that activation of pro-inflammatory cytokines in the brain of exposed mice. Whether this is due to the fraction of combustion NPs remains to be investigated (Borm et al. 2006).

4.3.5 Reproduction toxicology

The potential of NPs to enter the blood implies the possibility of transfer to reproductive organs and of transfer across the placenta leading to embryotoxicity (Fujimoto et al. 2005).Data addressing the distribution of NPs to the reproductive cells are, as yet, unavailable. In addition, no clear data are

available identifying the distribution of NPs in the fetus. There is a need to understand the toxicokinetics of NPs as it relates to placenta and foetus and teratogenicity of NPs in general (Tran et al. 2005)

4.3.6 Mutagenicity

It seems that on the cellular level, barriers such as cell membranes do not constitute obstacles for NPs. A great number of interactions with cell components are conceivable for particles penetrating into a cell. However, the health implications of such possible interactions are still unknown (UBA, 2006). During interaction with biological tissues, various factors are important such as material composition, electronic structure, bonded surface species (e.g., metal-containing), surface coatings (active or passive), and solubility, including the contribution of surface species and coatings and interactions of NP with other environmental factors (e.g., UV activation; (Nel et al. 2006). Recently, SCENIHR (SCENIHR 2006) concluded that there is a clear need for validated *in vitro* assays for nanoparticle evaluation, including assays with meaningful endpoints for genotoxicity. Also important to note is that carcinogenic effects for some particles are a consequence of inflammation which would not be detected by "classical" assays of genotoxicity (Donaldson et al. 2007).

4.3.7 Allergenicity (sensitization)

A further issue relevant for the exposure to NPs via food relates to the interactions of NPs with other food components. For example food containing NPs with actively charged surfaces can absorb biomolecules as they pass through the GI tract (Govers et al. 1994). These so called 'Trojan horses' (Lomer et al. 2002) may transport toxins into the intestinal mucosa, resulting in changed exposure of the cellular lining of the intestine (Borm and Kreyling 2004). It has been show that this altered presentation can affect the local immune response, a mechanism suggested to be related to Crohn's disease (Lomer et al. 2002). This latter mechanism might relate to possible (local) intolerance and/or allergenic responses of NPs. In addition the surface properties (e.g. coatings) are important determinants for the active uptake of encapsulates, but might also be a reason for concern. For example lectins used for coatings are highly immunogenic, can be cytotoxic or induce inflammatory responses and gastrointestinal irritation (des Rieux et al. 2006; Gabor et al. 2004).
Even for normal chemicals, risk assessors are confronted with a lack of knowledge on the induction of food allergy and the type of exposure required to induce such responses. The possible adjuvant activity of NPs described above however introduces additional uncertainty. If a food allergy to a NP is established, labeling and traceability is considered to be critical to anticipate and exclude possible sources for such potential allergens (Kroes et al. 2002).

4.3.8 Summary /interpretation of toxicology

In short, the results of the available toxicity studies indicate that acute oral exposure to large amounts may induce toxic effects mainly in the liver and kidney, but also in the blood, heart and spleen. No information on the chronic toxicity after low dose oral exposure is available (SCENIHR 2006). Information from toxicity studies with other exposure routes indicate that several systemic effects on different organ systems may occur after exposure to NPs, including the immune, inflammatory and the cardiovascular systems. Effects on the immune and inflammatory systems may lead to oxidative stress and/or activation of pro-inflammatory cytokines in the lungs, liver, heart and brain. Effects on the cardiovascular system may include pro-thrombotic effects and adverse effects on the cardiac function (acute myocardial infarction and adverse effects on the heart rate). Furthermore, genotoxicity, and possible carcinogenesis and teratogenicity may occur, but no data on these latter endpoints are available yet.

Despite all previous research done on NP, scientific knowledge on the potential toxicology of NP is limited. NPs may have an increased toxicological profile when compared to their conventional counterparts. Furthermore, the question arises whether the standard battery of tests in protocol toxicology is useful for the detection of the different toxicological profile of NP as not all effects identified above are routinely studied in these tests.
Future research should focus on validated *in vitro* assays for nanoparticle evaluation, including assays with meaningful endpoints for genotoxicity.

4.4 Exposure assessment of nanoparticles

Human beings are continuously exposed to natural and unintentionally produced NPs (e.g. fine dust particles, sand dust). Exposure assessment includes the entire life cycle of nanomaterials from synthesis to disposal. Likely, inhalation is the most important route of exposure to these NPs. If expressed in number of particles, per breathe ca $4x10^6$ particles are inhaled, of which more than half remains in the lungs (Engineering. 2004). Direct oral exposure via food to NPs is estimated to be 10^{12}-10^{14} micro- and NPs per person per day (mainly silicates and titanium dioxide) (Lomer et al. 2004; Lomer et al. 2002). Indirect exposure of the gastrointestinal tract can also be expected due to clearance of in the lungs deposited NPs via the mucociliary escalator (coughing up and swallowing of inhaled NPs). The contribution of the latter exposure route to the total amount of NPs, however, has not been quantified. In the two sections below relevant food exposure sources will be explored followed by an analysis of special requirements that NPs pose on the exposure assessment as currently employed for normal chemicals.

4.4.1 Data requirements and methodology

Exposure assessment is defined as the qualitative and /or quantitative evaluation of the likely intake of biological, chemical or physical agents via food as well as exposure from other sources if relevant (WHO, 1997). To perform an exposure assessment the following type of information is needed (Kroes et al. 2002):

- Which substances are present in which amounts in a given food and/or the diet in general and what affects their levels and characteristics, especially their biological activity?
- How much of the foods containing these substances are consumed and what is the consumption of potentially relevant risk groups, including high and frequent users?
- What are the conditions and the probabilities of consuming occasionally or regularly high amounts of such foods which at the same time contain high levels of the substance(s) in question?

Basically, the principle of exposure assessment of NPs (via food) will be comparable to the exposure assessment of 'normal' chemicals. However some aspects do require specific attention, these will be highlighted below.

Amount of NPs present in food commodities

A central aspect of exposure assessment is the determination of the amount and characterization of the NPs (or normal chemicals) present in the food as consumed (see chapter 3). Issues like food sampling and variability within composite samples and variation in concentrations between samples are not different from the exposure assessment of normal chemicals.

For an exposure assessment of nanoscale delivery systems loaded with bioactive compounds or bioactive compounds themselves in nanoscale formulations, both the amount of bioactive compounds within the capsules as well as the free form in the food matrix has to be determined. For this, the analytical isolation, detection and characterization procedures need to be designed to meet these requirements.
The presence of NP in the food matrix might result in increased bioavailability of substances (both nutrients and contaminants) normally present in the food. This needs to be considered when the presence of NPs in food is known.It will not always be feasible to measure chemicals and NPs in the food matrix in the consumable form. If chemicals are measured at an early stage of the food chain, effects of processing should be considered in exposure assessment (Kroes et al. 2002). The influence of processing at the stage of household preparation is, like in the exposure assessment of normal chemicals, also important (Kroes et al. 2002) for NPs. However, the default or database derived processing factors that are being used for determination of exposure assessment of normal chemicals when the exact effect of processing is unknown, (e.g. pesticides (JMPR)), are not (yet) available for NPs.

Consumption
Various sources of consumption data are currently utilized ranging from standardized food baskets used in pre-marketing authorizations to household or individual dietary surveys used in post-marketing studies (Kroes et al. 2002). There are no additional requirements for consumption data to be able to perform an exposure assessment for NPs that can be expected to be present in a variety of products (e.g residues of nanopesticides, processing aids or packaging materials). The use of NPs as additive or in special foods (novel foods or supplements) might require additional data on consumption of these special foods, because this information is generally lacking in the regular consumption databases. This is of course a general problem for exposure assessments, but more prominent in evaluating NPs because these particles are incorporated frequently in food supplements (see chapter3).

Intake calculations
The last step in performing the exposure assessment is the integration of food consumption and amount of chemicals or NPs present in food. Usually one of the following three approaches is applied for this integration of data: 1) point estimated; 2) simple distributions; 3) probabilistic analyses (Kroes et al. 2002). In the end the consumer exposure will be compared to a to a toxicological reference value e.g. tolerable daily intake, acceptable daily intake or acute reference dose or nutritional reference values like recommended daily intake or upper safe intake levels. These reference values are lacking for NPs and need to be established.

4.5 Risk Assessment of nanoparticles

As with conventional chemicals, risk assessment will be the basis of assessing and regulating nanomaterials (in food) to protect human health and the environment.

4.5.1 Establishing health-based guidance values
The last step in the hazard characterization is the setting of health-based guidance values such as acceptable daily intakes (ADIs) for food additives and pesticide residues, and tolerable daily intakes (TDIs). These values are based on the data from (animal) studies as indicated above. Reference points

(e.g. the no-observed-adverse-effect-level or benchmark-dose-level) for the most critical effect of a substance forms the reference point for the risk assessment. This is a general approach for all substances either being in a conventional form or at a nano-sized scale. In the case of health-based guidance values some issues require special attention.
For NPs, dose-response relationships will have to be analyzed case by case using different dose-describing parameters. As already mentioned before, the size of NP is not the only critical factor to explain the novel properties of NP but also the overall number (e.g. total surface area) may be relevant. So simply using mass in dose metrics will not be sufficient. However, up till now it has not been possible to establish a single alternative dose-describing parameter that best describes the dose and the observed dose response relations in toxicological tests.
A second issue is the biological activity of NPs present in food. The health-based guidance values are based on toxicological studies performed with NPs with a given bioavailability. NPs are often introduced to enhance the bioavailability of either themselves, or of the bioactive compounds loaded into them (nanoencapsulates) of to affect the uptake of other nutrients (or contaminants) present in the food. If by some means the bioavailability is changed (increased), this may affect the outcome of the toxicity studies and thus the calculated guidance values. Extrapolation of an health-based guidance value between formulations with different bioavailability might not be possible. Ultimately, this might require setting of separate values depending on the formulation.

4.5.2 Combining hazard and exposure

For risk assessment both information on exposure as well as on the (intrinsic) toxicity (hazard) of a compound is required. Determining potential consumer exposure is first of all important to assess the potential risk for consumers. Keeping in mind Paracelcus quote *"Alle Ding sind Gift und nichts ohn Gift; allein die Dosis macht, das ein Ding kein Gift ist"*(All things are poison and nothing (is) without poison; only the dose makes that a thing is no poison). Thus the dose of NPs present in food needs to be determined. As stated earlier, engineered NPs can have novel toxicological properties, that are attributed to their small size, chemical composition and surface structure (Nel et al. 2006). Since it has not been possible to establish a single dose-describing parameter that best describes the toxic effect , NPs should be characterized as completely as possible (Oberdorster et al. 2005a; Powers et al. 2006; Thomas and Sayre 2005). A further complicating factor is that the physico-chemical characteristics of NPs are highly depending on the matrix in which they are present (Oberdorster et al. 2005a; Powers et al. 2006). Thus NPs needs to be characterised in the food matrix (e.g. *in situ*).
Because of these uncertainties both in hazard assessment of NPs and in the exposure assessment, it is very important for risk assessors to have access to a clear description of the analytical methods that were used to determine the physicochemical properties of the respective NP, to have access to the (raw) experimental data and a sound description of the statistical procedure used to analyze the data. Only then a reliable assessment of the NPs can be performed and only then the results of the safety assessment can be used to model NP properties in silico (Powers et al. 2007), or compare results with those of non-nano counterparts. Another problem may be the comparison of different particle sizes and forms of the same compound. This problem is given in by the fact that proper dose metrics are still lacking.

5 Review of food related legislation and guidance documents related to nanotechnology in food

Different engineered nano-scaled materials are or will be used in food products, will end up in food as residues or contaminants and, are or will be used in products in contact with food, this is indicated in detail in chapter 3. Examples are nanoscaled food additives, micronutrients or essential elements, residues of nanoscaled pesticides or veterinary drugs, or 'intelligent' nano-contituents of food packaging material. Also nanoscaled encapsulates to deliver micronutrients or other food components at the 'right' sites in the body are being developed.
For most of these substances legislation already exists for the same materials of conventional chemicals. For 'regulated' substances, substances that are not allowed on the market unless they have been authorized, in general a safety assessment will have been made before market entry. In order to conduct a safety assessment sufficient toxicological hazard information should be made available by the producers of the substances. This will also be the case for nanosubstances subject to authorization. Another aspect is the monitoring of nanoparticles (NPs) in the food chain. Also for the official monitoring legislation is already existent.
In the existing legislation however, no reference is made to nanotechnology. In the next paragraphs the legislation concerning the use of NPs in relation to food, and the legislation concerning the official monitoring will be discussed. Potential gaps in the regulation are presented. Furthermore possible necessary modifications in legislation with respect to nanotechnology products are given.

5.1 Methodology

In this project only overarching reviews were used to present an outline of regulations that are applicable in the case of use of engineered NPs in or in contact with food, and residues of or contamination with nanomaterials of food. Especially the review presented by Frater (Frater et al. 2006) and the regulatory review of the Food Standards Agency of the UK (FSA March 2006) were used intensively. The original legislative texts were examined only occasionally due to time and money constraints. If European legislative texts were consulted, the consolidated versions as presented on the Eur-lex website were used.

5.2 Discussion of food related legislation and guidance documents

Most of the legislation on food in the Netherlands is based on European Directives and Regulations. The following sections will describe briefly the regulations and the potential gaps in the legislation on food when NPs are considered.

5.2.1 The General Food Law.

For the purpose of the European General Food Regulation ((GFL) (EC/178/2002.)) food or foodstuff means (article 2) 'any substance or product, whether processed, partially processed or unprocessed, intended to be, or reasonably expected to be ingested by humans'. The GFL therefore also applies to food substances/products of nano-size expected to be ingested by humans.
According to the GFL all foods placed on the Community market must be safe, or as stated in article 14 sub 1: *'Food shall not be placed on the market if it is unsafe'*. Unsafe is defined as*' injurious to health'*

or *'unfit for human consumption'*. In making an assessment of food safety article 14 requires to take into account among others probable immediate, short term and/or long term effects on the consumer and subsequent generations. The GFL stipulates that it is the responsibility of 'food business operators' to ensure that their foods satisfy the requirements of food law. Furthermore the food business operator should be able to trace one step up and one step down 'any substance intended to be, or expected to be, incorporated into a food (article 18). To facilitate traceability food should be adequately labeled or identified (article 18 sub 4).
In paragraph 18 and article 6 of the regulation it is emphasized that in decision making, scientific risk assessment should be central. The precautionary principle is laid down in this regulation in article 7. This article stipulates that if after assessing the available information a possibility of harmful effects on health is identified but scientific uncertainty persist, risk management measures to ensure a high level of health protection may be adopted, pending gathering and developing further scientific information for a more comprehensive risk assessment.

Items to be considered: General Food Law

- The assessment of safety for some of the NPs that are to be used in food products will not be possible due to a lack of knowledge on likely effects of these NPs. A lack of data on safety of NPs may provoke the precautionary principle. The management measures adopted in this case should however be among others 'proportionate', and should be reviewed within a reasonable period of time (article 7 sub2).
- A requirement in the GFL is that member states should monitor to verify if the requirements of food law are fulfilled by food business operators (article 17). Also the European Food Safety Authority should, according to article 34, establish monitoring procedures to identify emerging risks. The monitoring of NPs will require the development of new analytical detection and confirmation techniques.

5.2.2 Novel food and novel food ingredients

The Novel Food Regulation (EC/258/97) concerns the placing on the European market of novel food and novel food ingredients. Novel is defined in this Regulation as *'not hitherto been used for human consumption to a significant degree within the Community'*. With 'not hitherto' May 15, 1997 is meant, the date the regulation came into force. Only 'novel' foods are regulated, furthermore the food and food ingredients must fall in one or more of the following categories (article 1):
- food and food ingredients with a new or intentionally modified primary molecular structure;
- food or food ingredients consisting of or isolated from micro-organisms, fungi or algea;
- food and food ingredients consisting of or isolated from plants, or isolated from animals, except for foods and food ingredients obtained by traditional propagating or breeding practices and having a history of safe use;
- foods and food ingredients to which has been applied a production process not currently used, where that process gives rise to significant changes in the composition or structure of the foods or food ingredients which affect nutritional value, metabolism or level of undesirable substances.

The Regulation does not apply to food additives and flavorings, and extraction solvents used in the production of foodstuffs, as for these substances, in most cases, other legislation applies.
Novel foods and novel food ingredients must not present a danger for the consumer, mislead the consumer or differ from foods or food ingredients which they are intended to replace to such an extent that their normal consumption would be nutritionally disadvantageous for the consumer (article 3).

Before a novel food or novel food ingredient is authorized a safety and nutritional assessment is made by the member state where the novel product will placed on the market for the first time. The quite extensive data requirements for this assessment are laid down in Commission Recommendation 97/618/EC.

Items to be considered: Novel Foods

- The Novel Food regulation does not address the size or shape of novel food ingredients. It does however address 'production processes not currently used' so nanotechnology as such is likely to fall under the regulation because of its novelty. It is not clear if the use of NPs in foods that are already on the market makes these foods 'novel' and thus requiring authorization. If 'old' ingredients are marketed in future in nano-sized forms, it is not certain that this regulation will apply.
- Article 3(4) of the regulation says that when certain food components are 'substantial equivalent' to their conventional counterparts they can be treated in the same manner as their counterparts. Only the 'equivalency' has to be proven. It is likely that some engineered NPs will be 'equivalent'. However article 3(4) only applies to certain categories of food and food ingredients, engineered NPs do not fall into the categories mentioned thus far in this regulation (the Regulation is under revision at this moment however).

5.2.3 Food additives, enzymes, flavorings and processing aids

In the European Union food additives are not allowed to be present in food unless they are authorized for use. According to European legislation on food additives, consisting of a framework Directive (89/107/EEC) and three specific Directives (94/36/EC (colors), 94/35/EC (sweeteners) and 95/2/EC (food additives other than colors and sweeteners)) and their amendments, food additives may only be authorized if there is a technological need for their use, if they do not mislead the consumer and if they do not present a health hazard to the consumer. Lists of permitted additives, the foods in which they can be used and, if necessary, maximum levels of use are present in the annexes of the additive directives. If no quantitative limits are set the maximum use of additives should still be limited by the necessity to achieve the desired technological effect (*quantum satis*). Furthermore additives must comply with specific purity criteria laid down in other directives.
So, before authorization ample information concerning the physico-chemical properties and toxicity of the additive have to be provided by the producer. Or as is stated in Annex II of 89/107/EC: *'to assess the possible harmful effects of a food additive or derivatives thereof, it must be subjected to appropriate toxicological testing'*. The safety assessment for food additives is nowadays performed by EFSA (panel on dietetic products, nutrition and allergies), previously by the Scientific Committee on Foods (SCF).
The scope of the food additive framework Directive 89/107/EC only covers enzymes used as food additives and not the use of enzymes as processing aids. Under 95/2/EC the use of two enzymes as food additives are allowed: an invertase and a lysozyme.
A size restriction ('not less than 5 µm') is, up till now, laid down for only two authorized food additives: microcrystalline cellulose and powdered cellulose, for safety reasons (96/77/EC). Form of the additive played a role in the authorization of titaniumdioxide. Originally only the anatase form was authorized. The rutile (platelet) and anatases forms of titanium dioxide are similar chemically but differ in their crystalline structure and light reflectance. The SCF concluded in her safety assessment on the rutile form that on the basis of a new bioavailability study in which different rutile and the anatase form were given orally to rats, that the bioavailability of the different form was essentially the same, and that the

toxicological database for the anatase form would be applicable to either form (EFSA 2004). Subsequently also the rutile form was named in a Directive, member states should include the rutile form in their legislation on food additives before April 10 2007 ((2006/33/EC.)).
Flavoring substances are up till now regulated by a separate Directive, Council Directive 88/388/EEC. In Directive 88/388/EEC a definition of 'flavoring' is given. Flavorings can be derived from plant and animal species or by chemical synthesis. The flavorings made by chemical synthesis are subdivided in this Directive in 'nature identical' and 'not chemically identical to a substance naturally present'. For certain undesirable substances (like safrole, coumarin, thuyone) present in foodstuffs due to flavoring or food ingredients maximum levels are set for some specified foods (Annex II). These substances may however not be added as such to foodstuffs or flavorings, so the mass limits set here are not a problem. The food additives Directives do not cover processing aids like most of the enzymes used and extraction solvents.
In the near future the European legislation on food additives, enzymes and flavorings will change. Use of enzymes will be covered by the future legislation. Proposals for Regulations on these issues (COM (2006) 425 final, COM (2006) 428 final, COM (2006) 427 final) were published July 2006, as was a proposal for a Regulation establishing a common authorization procedure (COM (2006) 423 final). Once accepted by the European Parliament and Council these Regulations will replace the current Directives.
No European proposal for a Regulation or a Directive on processing aids (other than enzymes) is formulated yet.

Items to be considered: Food additives

- On new ‘nano-additives’ existing food additives legislation will apply as it is likely that the nano-additives that are being developed will fall within the definition of a food additive (article 1 of 89/107/EEC) and will be used for the same reasons e.g. needs as the food additives authorized today. As for the toxicity tests to be performed, as stated previously under 'hazard characterization' (chapter 4.2 and 4.3), dose metrics should include information on the nano-properties of the substance.
- It is not clear if when an already authorized food additive is reformulated in nano-size this means that it is a new additive. However all authorized food additives must according to point 4 in Annex II be kept under continuous observation and must be re-evaluated whenever necessary in the light of changing conditions of use and new scientific information.
- In the Directives on purity criteria for food additives properties related to particle size should be included.
- A nano-flavor can be 'chemically identical' to a natural flavor but have a different toxicity profile due to properties related to its-nano-size.
- It is likely that NPs for use in food processing are being developed that fall outside the scope of the Directives and the future Regulations
- Although particle size was evaluated when authorizing two additives, in the 'purity criteria' Directives for food additives, size (with all its dimensions) is not mentioned (nor size distribution).

5.2.4 Food enrichment

A new European Regulation on the addition of vitamins and minerals and certain other substances to food has recently been published (EC/1925/2006.). It shall apply from 1 July 2007. In Annex I and

Annex II of this regulation vitamins and minerals (and the forms of the vitamins and minerals) are listed which may be added to food. The regulation requires that the nutrient added is in a bio-available form. Purity criteria for the vitamin formulations and mineral substances in the annexes shall be adopted in the near future. Addition to non-processed food and alcoholic beverages is prohibited. If a vitamin or mineral is added it is obligatory to provide for nutrition labeling. The regulation aims to harmonize the market of supplemented foods in the EU and to protect consumers from ingesting quantities of vitamins, minerals and other substances that could harm the health of the consumer. Most remarks in the next paragraph on food supplements (that are not copied here) are also applicable here. A difference however is that in EC/1925/2006 monitoring is mentioned, for monitoring nano-sized nutrients added to food analytical methods need to be developed that can provide information on the size of nutrients present.

5.2.5 Food supplements

Directive 2002/46/EC aims to harmonize the rules for marketing food supplements in the EU. A food supplement is defined as (article 2): *'Food supplements' means foodstuffs the purpose of which is to supplement the normal diet and which are concentrated sources of nutrients and other substances with nutritional or physiological effect, alone or in combination, marketed in dose forms, namely forms such as capsules, pastilles, tablets, pills and other similar forms, sachets of powder, ampoules of liquid, drop suspensing bottles and other similar forms of liquids and powders designed to be taken in measured small unit quantities'*.

With nutrients vitamins and minerals are meant. In Annex I of the directive vitamins and mineral substances are listed which may be used in the manufacture of food supplements, Annex II contains the forms such as thiamin hydrochloride and thiamin moninitrate (vitamin B1), cyanocobalamin and hydroxycobalamin (vitamin B12), potassium fluoride and sodium fluoride (fluoride), and cupric carbonate, cupric gluconate, cupric citrate, cupric sulphate and copper lysine complex (copper). Purity criteria for the substances in Annex II will be adopted, unless purity criteria are already set in other Community legislation when the same substance is used for other purposes than covered by this directive. Member states may ban supplements from their markets that contain vitamins and minerals not included in Annex I or in forms not included in Annex II (article 4sub7). They may however also allow supplements containing vitamins and minerals and forms not listed in the annexes that were used in one or more food supplements marketed in the Community before 12 July 2002 and for which the EFSA has not given an unfavorable opinion. July 2007 the Commission will have prepared a report concerning the use of substances other than vitamins and minerals in food supplements.

Producers of food supplements should take into account when choosing the amounts of vitamins and minerals present in food supplements per daily portion, the upper safe limits of vitamins and minerals as established by scientific risk assessment (taking into account the varying degrees of sensitivity of different consumers groups) and the intake of vitamins and minerals from other dietary sources. The manufacturer should declare on the label of a food supplement the amount (in mg, µg) of nutrients or other substances present, the amount per portion and expressed as a percentage of the reference value (e.g. the recommended daily intake).

For minerals or trace elements particle size may be a determinant of oral absorption. Rohner and colleagues ((Rohner et al. 2007)) showed that lowering the size $FePO_4$ (a poorly soluble iron compound) to nano size (10.7 NP) with a spherical structure increased its bioavailability in rats. These authors also mentioned studies in which it was established that the acute toxicity of nanoscaled copper and zinc were higher than those of equivalent amounts of micro-copper and micro-zinc.

For some essential nutrients such as vitamins and minerals the gap between the recommended dose and the toxic dose is narrow. Overdosing of minerals and (fat-soluble) vitamins can lead to adverse health effects.

Items to be considered: Food supplements

- Nanosizing of especially minerals may improve their bioavailability. Adjustment of amounts in supplements should take into account the improved bioavailability to prevent overdosing.
- Purity criteria should include information on the size and form of the substance
- Established maximum intakes may have to be revisited if nano-sized nutrients with improved absorption characteristics are added to food or present in food supplements.

5.2.6 Materials coming into contact with food

Engineered NPs to improve food packaging materials are being developed. These will improve for instance the strength or the barrier properties of the materials. Also antimicrobial engineered NPs will be used in packaging to extend shelf life and improve food safety characteristics and many other 'active' and ‘intelligent NPs', intended to come into contact with food are in the pipeline.
All materials that come into contact directly or indirectly with food, including active and intelligent NPs, are subject to EC regulation (EC/1935/2004.). Active food contact materials and articles are defined as (article 2 sub 2a) *'materials and articles that are intended to extend the shelf-life or to maintain or improve the condition of packaged food. They are designed to deliberately incorporate components that would release or absorb substances into or from the packaged food or the environment surrounding the food'*. 'Intelligent' means materials and articles which monitor the condition of packaged food or the environment surrounding the food (article 2 sub2b). Active and intelligent materials and articles need to be authorized (article 4 sub2) and should be adequately labeled (article 4 sub5-6). As according to the Regulation the active and intelligent materials shall be considered as ingredients (article 4 sub2), also the Directive on labeling of foodstuffs (2000/13/EC) applies.
EC/1935/2004 requires among others that packaging materials and other articles coming into contact with food under normal or foreseeable conditions of use do not transfer their constituents in quantities that could endanger the consumer's health. Moreover the materials and articles should not bring about an unacceptable change in the composition of the food or deterioration in its organoleptic properties (article 3 sub-abc). As of 27 October 2006 business operators should be able to trace their packaging materials and articles one step up and one step down (article 17). Here again, traceability requires labeling or other measures to assure tracing.
The EU has adopted more specific measures for plastic materials (2002/72/EC), regenerated cellulose (93/10/EC) and ceramics (84/500/EC). These specific directives contain positive lists of substances that may be used in food contact materials. For each category of substances restrictions on the migration are mentioned. Maximum migration levels are expressed in mass of substance per mass or volume of packaged food (.e.g. high = 5-60 mg/kg/food, low = <0,05 mg/kg/food).
Migration of active and intelligent NPs into foodstuffs is a likely occurrence. Depending on the migration level of the NPs more or less safety data should be supplied to EFSA. The safety assessment is made by EFSA.

Items to be considered: Food contact materials

- Expressing the migration levels in mass per mass or volumes does not take into account the possibility of changing toxicity profiles with the lowering in size of a substance. Migration level cuts offs in the legislation may therefore not be adequate for NPs.
- Food contact materials with NPs falling in the low migration category for which a limited dataset is required may be authorized without a sufficient assessment of safety for man and the environment.
- Because of the present lack of safety data also here the precautionary principle may be used to prevent NPs from being used in food contact materials. If member states suspect that a contact material endangers human health they may suspend the application within its territory (article 18).

5.2.7 Other (contaminants, pesticides, veterinary drugs)

Due to the use of NPs in products like quantum dots or pesticide formulations foods may become contaminated with residues of the NPs. In de next sections attention will be paid to the legislation in the European Union concerning contaminants and residues. This because legislation in member states concerning contaminants and residues in food is to a large extent based on EU directives and regulations. The difference between 'residues' and 'contaminants' in EU law is that residues are the result of legal use of substances during food production whereas contaminants may be present in food as the result of their presence in the environment. The difference however is somewhat arbitrary, like for some older forbidden pesticides that are not used anymore but still contaminate food due to former usage.

5.2.7.1 Contaminants

EU regulation EC/1881/2006 sets maximum levels for certain contaminants in foodstuffs. Maximum levels for contaminants are set at levels that are reasonably achievable by following good agricultural, fishery and manufacturing practices. Also the risk related to the consumption of contaminated food is taken into account. Before maximum levels are set a risk assessment is made, nowadays in Europe by the EFSA scientific panel on contaminants. The result of a risk assessment is that maximum intake levels by humans are established, like Tolerable Daily Intakes (TDI) or (provisional) Tolerable Weekly Intakes (pTWI). The in vitro and in vivo studies used to evaluate the risk of contaminants are to a large extent published in scientific literature or performed by laboratories related to governments. From the description of these studies it is sometimes hard to derive the precise substance tested (sometimes no specification of the materials tested is given), information concerning size, three-dimensional structure or volume of the tested substance are always lacking.
Taking into account consumption figures and background levels in food, maximum levels are proposed. The maximum levels of contaminants in food are expressed as concentrations, e.g. mg substance per kg food. The size of a substance is not included in the threshold value. NPs however may or will have different toxic properties than their non-nanoparticle counterparts. However also for 'normally' sized substances thresholds can be just an estimation of 'safe' limits. For example: cadmium (Cd) can be present in different forms like metallic Cd (not soluble), Cd-sulfide (low solubility) and Cd-chloride (very soluble). The oral LD50s in mice for Cd-sulfide and Cd-chloride are 1200 mg/kg bw and 94 mg/kg bw respectively (Bellinger et al. 2004). It is not easy to take into account the different properties of all the different Cd forms in the establishment of pTWI and limits in food. The maximum limits established in Europe only mention 'cadmium' meaning 'total cadmium' irrespective of the Cd-containing compound present. Also for mercury (Hg) and arsenic (As) for example the limits set for

total Hg and total As, and are not specified although it is known that the inorganic compounds of these heavy metals have a vastly different toxicity profile than the organic compounds.

Items to be considered: Contaminants

- Additional safety tests may be necessary to investigate if human health limits set (like TDIs, pTWIs etc) are still valid for nano-sized particle contaminants
- Maximum levels set today for certain contaminants in food may be inadequate to protect the consumer health due to increased toxicity of the nano-size version of the contaminants.

5.2.7.2 Pesticides used in crops

The placing on the market of pesticides used to protect plant or products of plants is regulated by Directive 91/414/EC. Before a substance is allowed on the market to be used as a pesticide a safety assessment is made. Once a substance is approved member states may authorize the use of the substance in plant protection products. The safety assessment is meant to assure that when plant pesticide products are used correctly, no harmful effects will occur in persons applying the pesticide, consumers or domestic animals. Furthermore an assessment is made of the impact on the environment. EFSA peer reviews the assessments made. The use of pesticides may result in residues in plant products. Maximum Residue Levels (MRLs) are therefore set at a level in agreement with good agricultural practice (GAP) and taking into account human health based safety limits (ADIs). To set MRLs data from residue trials are evaluated. At this moment 4 EU directives contain the harmonized EU MRLs. Besides EU MRLs member states have national MRLs for substances not harmonized yet. In the near future there only will be EU MRLs in one Regulation (EC/396/2005).

Items to be considered: Pesticides

- Inclusion of nano-particles in pesticides where the non-nano form has already been authorized may require additional safety testing and a new approval
- In the assessment of the residue data provided by the producer, ample consideration should be given to the (range of) formulations as residue formation may be different due to the use of nanotechnology.
- Nano-sized active constituents may have different toxicity and residue profiles than the same normal sized constituents, re-assessment of ADIs and MRLs may be necessary.

5.2.7.3 Veterinary drugs

Veterinary drugs are not allowed to be used unless they are registered. The registration for the use of a veterinary medicinal product is granted at the national level. However procedures, data requirements etc for registration are harmonized to a very large extent within the EU by Directive 2001/82/EC (2001/82/EC). Furthermore before any pharmacologically active substance is allowed on the EU market for use in food producing animals, maximum residue limits (MRLs) should be established on EU level (EEC/2377/90.). In human medicine developments on nanosubstances to enhance efficacy of drugs or improve diagnosis are well underway. In medicines used for treatment of animals NPs may also be used in future. The use of NPs as excipients or as active constituents to enhance efficacy may also result in alterations in the absorption, metabolism, distribution or excretion (ADME) of the active constituent(s) of the drug. Plus the toxicity, pharmacological and if applicable, antimicrobial profile of a nano-sized active constituent may be different from the evaluated normal size variant. So, established ADIs and MRLs may have to be revisited, or set for the nano-sized variants of active constituents. To this end data

concerning the characteristics of the nano-contituents in addition to those of the non-nano variant are a necessity. This is especially the case for nano-encapsulation of active constituents to improve drug delivery at target sites. In this case also the toxicity profile of the capsule could be of interest.

Items to be considered: Veterinary drugs

- If a nanomaterial is incorporated in an already registered veterinary medicinal product it is not sure if the product should be re-registered. Alterations in the composition of a product should be brought to attention of the registration authorities. An alteration in size in any of the substances used as constituents of a veterinary drug does not result in a change in chemical composition. However due to altered characteristics of nano-constituents compared to their non-nano equivalents, products should not be considered to 'essentally similar' without any confirming data.
- Nano-sized active constituents may have different ADME and toxicity profiles than the same normal sized constituents, re-assessment of ADIs and MRLs may be necessary.

5.2.8 Not food related legislation - REACH

Present legislation on existing and new chemical substances and the new Regulation on chemicals (REACH, EC) do aim to obtain information on dangers of substances. Information should be supplied by the producer and the review of the data included in a notification ensures that the substance is labeled for supply, and that there is information to take the appropriate measures to reduce risk during use and disposal. Present Directives differentiate between existing (this is defined as: placed on the market before September 1981, and listed in EINECS (European Inventory of Existing Commercial Chemical Substances)) substances and new substances. Data requirements for new substances are more stringent. The new regulation REACH introduces testing requirements not only for the new substances but also for the ones previously called 'existing'. It is up to the producer to determine whether or not the nano-substance he produced and which is on the EINICS list should be considered as existing (no new data) or new. In the old Directives as well as in REACH also the quantity of a substance produced determines the set of data on safety that has to be provided by the producer. These mass triggers may not be appropriate for nanochemicals.
Additional safety testing and evaluation of nano-versions of chemicals for which safety evaluations were already made, is not required in the legislation on chemical substances, since the nanoscale form does not constitute a change in the chemical structure. In describing the chemical identity (CAS nr, EINECS nr) of substances no size-properties and size distributions of the chemical are demanded in the legislation. If no information on the presence of NPs in products is passed through the chain of production, transport, use and disposal, NPs will end up in the environment without anyone knowing it. If the nanomaterial turns out to be a hazardous substance to hold the producer responsible and trace any other introductions into the environment will be very difficult. Nano-substances may eventually end up in the food chain, and be regulated as contaminants. Adequate safety testing, including investigations on the fate in the environment, before use of the nanomaterial, may prevent the entrance of harmful nano-substances as contaminants in food (and feed).

Items to consider: REACH

- The adequacy of the quantity mass limits set in REACH in relation to nanochemicals
- The addition of an indication of particle size characteristics to the chemical name or number, to differentiate the nano from the normal sized substances, and to make an additional safety assessment for nano-varieties obligatory
- Reassessment of the human health limits set (ADI, OEL, etc) to include or set separate limits for nano-scale substances if necessary
- Monitoring of exposure requires valid and suitable methods; exposure may have to be expressed in units other than mass, certainly for carbon nanotubes and NPs in the form of fibres.

5.3 Overall conclusions

1) In authorization procedures legislation, guidelines and guidance documents describe how and which toxicity tests should be performed. For NPs it is necessary to include herein that the physico-chemical parameters should include information on e.g. particle size, particle form, surface properties and other size properties that may influence the toxicity of the substance *(see chapter 4.1)*. Furthermore appropriate dose metrics to use in the hazard characterization should be developed *(see chapter 4.2 and 4.3)*. Methodological changes in safety test protocols may be required to account for toxicity mechanisms of NPs not found in 'normal size' materials *(see chapter 4.1)*. To assess the exposure of the food consumer to NPs it may also be necessary to investigate what the most appropriate dose metric would be *(see chapter 4.4 and 4.5)*.
2) For all substances for which an authorization procedure including toxicity testing has already been established before introduction onto the market, there is no need for new legislation in case of nanosizing of the substances, however re-evaluation of nanosized substances may be necessary. Nano-sized materials should therefore always be considered as 'new' (in all the legislation where a difference is made between 'existing' and 'new' substances).
3) Adjustments of legislation, guidelines and guidance documents concerning the testing of the substance are necessary, especially concerning the physico-chemical characterization of the tested substance.
4) Thresholds or limits set in different legislation that are expressed in concentrations of particular substances or via percentages or weight (e.g. maximum use level of an food additive, maximum limit for a contaminant, migration thresholds for food contact materials, and others) do not take into account the difference in properties of nanosized and non-nanosized substances. So thresholds or limits set already may be not appropriate for nanosized variants of the particular substances.

6 Reference List

52/2/EC. European Parliament and Council Directive 52/2/EC of 20 February 1995 on food additives other than colours and sweeteners Official Journal of the European Union L 61, 18. 3. 1995, p. 1

84/500/EC. Council Directive 84/500/EC of the European Parliament and of the council of 15 October 1984 on the approximation of the laws of the Member States relating to ceramic articles intended to come into contact with foodstuffs. Official Journal of the European Union L 277, 20.10.1984, p12

88/388/EEC. Council Directive 88/388/EEC of the European Parliament and of the council of 22 June 1988 on the approximation of the laws of the Member States relating to flavourings for use in foodstuffs and to source materials for their production. Official Journal of the European Union L 184, 15.7.1988, p. 61

89/107/EEC. Council Directive No 89/107/EEC of the European Parliament and of the council of 21 December 1988 on the approximation of the laws of the Member States concerning food additives authorized for use in foodstuffs intended for human consumption. Official Journal of the European Union L 40, 11.2.1989, p. 27

93/10/EC. Commission Directive 93/10/EEC of the European Parliament and of the council of 15 March 1993 relating to materials and articles made of regenerated cellulose film intended to come into contact with foodstuffs. Official Journal of the European Union L 93, 17.4.1993, p. 27

94/35/EC. European Parliament and Council Directive 94/35/EC of the European Parliament and of the council of 30 June 1994 on sweeteners for use in foodstuffs. Official Journal of the European Union L 237, 10. 9. 1994, p. 3

94/36/EC. European Parliament and Council Directive 94/36/EC of the European Parliament and of the council of 30 June 1994 on colours for use in foodstuffs. Official Journal of the European Union L237, 10.9.1994, p13

96/77/EC Commission Directive 96/77/EC. of the European Parliament and of the council of 2 December 1996. laying down specific purity criteria on food additives other than colours and sweeteners. Official Journal of the European Union L 339, 30.12.1996, p. 1

2002/46/EC. Regulation (EC) No 2002/46 of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements. Official Journal of the European Union L 183, 12.7.2002, p. 51-

2000/13/EC. Directive 2000/13/EC of the European Parliament and of the Council of 20 March 2000 on the approximation of the laws of the Member States relating to the labelling, presentation and advertising of foodstuffs. Official Journal of the European Union L 109, 6.5.2000, p 29-.

2001/82/EC. Directive 2001/82/EC of the European Parliament and Council of 6 November 2001 on the Community code relating to veterinary medicinal products. Official Journal of the European Union L 311, 28.11.2001, p 1-

2006/33/EC. Directive 2006/33/EC of the Commission of 20 March 2006 amending Directive 95/45/EC as regards sunset yellow FCF (E 110) and titanium dioxide (E 171). Official Journal of the European Union L 82, 21.3.2006, p10-.

Aprahamian M, Michel C, Humbert W, Devissaguet JP, Damge C. 1987. Transmucosal Passage of Polyalkylcyanoacrylate Nanocapsules as a New Drug Carrier in the Small-Intestine. Biology of the Cell 61(1-2):69-76.

Avella M, De Vlieger JJ, Errico ME, Fischer S, Vacca P, Volpe MG. 2005. Biodegradable starch/clay nanocomposite films for food packaging applications. Food Chemistry 93(3):467-474.

Ballou B, Lagerholm BC, Ernst LA, Bruchez MP, Waggoner AS. 2004. Noninvasive imaging of quantum dots in mice. Bioconjug Chem 15(1):79-86.

Bellinger D, Bolger M, Egan K, Feeley M, Schlatter J, Tohyama C. 2004. CADMIUM (addendum). Report nr 52.

Bontempo V, Baldi A, Cheli F, Fantuz F, Prod A, Politis I, Sc A, Carli S, Dell'Orto V. 2000. Kinetic behavior of three preparations of alpha-tocopherol after oral administration to postpubertal heifers. American Journal of Veterinary Research 61(5):589-593.
Borm P, Klaessig FC, Landry TD, Moudgil B, Pauluhn J, Thomas K, Trottier R, Wood S. 2006. Research strategies for safety evaluation of nanomaterials, part V: role of dissolution in biological fate and effects of nanoscale particles. Toxicol Sci 90(1):23-32.
Borm PJA, Kreyling W. 2004. Toxicological hazards of inhaled nanoparticles - Potential implications for drug delivery. Journal of Nanoscience and Nanotechnology 4(5):521-531.
Brown JS, Zeman KL, Bennett WD. 2002. Ultrafine particle deposition and clearance in the healthy and obstructed lung. American Journal of Respiratory and Critical Care Medicine 166(9):1240-1247.
Cagle DW, Kennel SJ, Mirzadeh S, Alford JM, Wilson LJ. 1999. In vivo studies of fullerene-based materials using endohedral metallofullerene radiotracers. Proc Natl Acad Sci U S A 96(9):5182-7.
Cansell M, Nacka F, Combe N. 2003. Marine lipid-based liposomes increase in vivo FA bioavailability. Lipids 38(5):551-559.
Carr KE, Hazzard RA, Reid S, Hodges GM. 1996. The effect of size on uptake of orally administered latex microparticles in the small intestine and transport to mesenteric lymph nodes. Pharm Res 13(8):1205-9.
Carrero-Sanchez JC, Elias AL, Mancilla R, Arrellin G, Terrones H, Laclette JP, Terrones M. 2006. Biocompatibility and toxicological studies of carbon nanotubes doped with nitrogen. Nano Lett 6(8):1609-16.
Chen HD, Weiss JC, Shahidi F. 2006a. Nanotechnology in nutraceuticals and functional foods. Food Technology 60(3):30-+.
Chen HHC, Yu C, Ueng TH, Chen SD, Chen BJ, Huang KJ, Chiang LY. 1998. Acute and subacute toxicity study of water-soluble polyalkylsulfonated C-60 in rats. Toxicologic Pathology 26(1):143-151.
Chen LY, Remondetto GE, Subirade M. 2006b. Food protein-based materials as nutraceutical delivery systems. Trends in Food Science & Technology 17(5):272-283.
Chen Z, Meng H, Xing G, Chen C, Zhao Y, Jia G, Wang T, Yuan H, Ye C, Zhao F and others. 2006c. Acute toxicological effects of copper nanoparticles in vivo. Toxicol Lett 163(2):109-20.
Coyle P, Philcox JC, Carey LC, Rofe AM. 2002. Metallothionein: The multipurpose protein. Cellular and Molecular Life Sciences 59(4):627-647.
DEFRA. 2005. A scoping study into the manufacture and use of nanomaterials in the UK.
Des Rieux A, Fievez V, Garinot M, Schneider YJ, Preat V. 2006. Nanoparticles as potential oral delivery systems of proteins and vaccines: a mechanistic approach. J Control Release 116(1):1-27.
Desai MP, Labhasetwar V, Amidon GL, Levy RJ. 1996. Gastrointestinal uptake of biodegradable microparticles: effect of particle size. Pharm Res 13(12):1838-45.
Donaldson K, Faus S, Borm PJA, Stone V. 2007. Approaches to the toxicological testing of particles. In: Donaldson K, Borm PJA, editors. Particle toxicology. London: CRC press Taylor and Francis group. p 299-316.
Donaldson K, Stone V, Clouter A, Renwick L, MacNee W. 2001. Ultrafine particles. Occup Environ Med 58(3):211-6, 199.
Duncan R, Izzo L. 2005. Dendrimer biocompatibility and toxicity. Adv Drug Deliv Rev 57(15):2215-37.
EC/178/2002. Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and the requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. Official Journal of the European Union L 31, 1.2.2002, p 1-.
EC/258/97. Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients. Official Journal of the European Union L 43, 14.2.1997, p. 1–6

EC/396/2005. Regulation(EC) No 396/2005 of the European Parliament and of the council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC. Official Journal of the European Union L 70, 16.3.2005, p1

EC/1881/2006. Commission Regulation (EC) No 1881/2006. of the European Parliament and of the council of 19 December 2006. setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Union L 364, 20.12.2006, p5

EC/1925/2006. Regulation (EC) No 1925/2006of the European Parliament and of the Council of 20 December 2006 on the addition of vitamins and minerals and of certain other substances to food. Official Journal of the European Union L404, 30.12.2006, p26-.

EC/1935/2004. Regulation (EC) No 1935/2004 of the European Parliament and of the Council of 27 October 2004 on materials and articles intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EEC. Official Journal of the European Union L 338, 13.11.2004, p 4-.

EEC/2377/90. Council Regulation (EEC) No 2377/90 of 26 June 1990 laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin. Official Journal of the European Union L 224, 18.8.1990, p 1-.

EFSA. 2004. . Opinion of the Scientific Panel on Food Additives, Falvourings, Processing Aids and materials in Contact with Food on a request from the Commission related to the safety in use of rutile titatnium dioxide as an alternative to the presently anastase form. Question N0 EFSA-Q-2004-103. Adopted on 7 December 2004. The EFSA Journal (2004) 163, 1-.

Elder A, Gelein R, Silva V, Feikert T, Opanashuk L, Carter J, Potter R, Maynard A, Ito Y, Finkelstein J and others. 2006. Translocation of inhaled ultrafine manganese oxide particles to the central nervous system. Environ Health Perspect 114(8):1172-8.

Engineering. TRSatRAo. 2004. The Royal Society and the Royal Academy of Engineering. Nanoscience and nanotechnologies: opportunities and uncertainties. London, UK .

Florence AT. 1997. The oral absorption of micro- and nanoparticulates: neither exceptional nor unusual. Pharm Res 14(3):259-66.

Florence AT. 2005. Nanoparticle uptake by the oral route: Fulfilling its potential? Drug Discovery Today: Technologies 2(1):75-81.

Frater L, Stokes E, Lee R, Oriola T. 2006. An Overview of the framework of current regulation affecting the development and marketing of nanoparticles. A report for the DTI. ESRC Centre for Business Relationships Accountability Sustainability and Society (BRASS). Cardiff University. December 2006. URN 06/220.

FSA. March 2006. Draft report of FSA regulatory review. Aew of potential implications of nanotechnologies for regulations and risk assessmen in relation to food.

Fujimoto A, Tsukue N, Watanabe M, Sugawara I, Yanagisawa R, Takano H, Yoshida S, Takeda K. 2005. Diesel exhaust affects immunological action in the placentas of mice. Environ Toxicol 20(4):431-40.

Gabor F, Bogner E, Weissenboeck A, Wirth M. 2004. The lectin-cell interaction and its implications to intestinal lectin-mediated drug delivery. Adv Drug Deliv Rev 56(4):459-80.

Geiser M, Rothen-Rutishauser B, Kapp N, Schurch S, Kreyling W, Schulz H, Semmler M, Im Hof V, Heyder J, Gehr P. 2005. Ultrafine particles cross cellular membranes by nonphagocytic mechanisms in lungs and in cultured cells. Environ Health Perspect 113(11):1555-60.

Gouin S. 2004. Microencapsulation: industrial appraisal of existing technologies and trends. Trends in Food Science & Technology 15(7-8):330-347.

Govers M, Termont D, Vanaken GA, Vandermeer R. 1994. Characterization of the Adsorption of Conjugated and Unconjugated Bile-Acids to Insoluble, Amorphous Calcium-Phosphate. Journal of Lipid Research 35(5):741-748.

Graveland-Bikker JF, de Kruif CG. 2006. Unique milk protein based nanotubes: Food and nanotechnology meet. Trends in Food Science & Technology 17(5):196-203.

Hardman R. 2006. A toxicologic review of quantum dots: toxicity depends on physicochemical and environmental factors. Environ Health Perspect 114(2):165-72.

HealthCouncilNetherlands. 2006 Health significance of nanotechnologies. The Hague: Health Council of the Netherlands, publication no. 2006/06.
Hillery AM, Jani PU, Florence AT. 1994. Comparative, quantitative study of lymphoid and non-lymphoid uptake of 60 nm polystyrene particles. J Drug Target 2(2):151-6.
Hillyer JF, Albrecht RM. 2001. Gastrointestinal persorption and tissue distribution of differently sized colloidal gold nanoparticles. Journal of Pharmaceutical Sciences 90(12):1927-1936.
Hoet P, Bruske-Hohlfeld I, Salata O. 2004. Nanoparticles - known and unknown health risks. Journal of Nanobiotechnology 2(1):12.
Hong SP, Leroueil PR, Janus EK, Peters JL, Kober MM, Islam MT, Orr BG, Baker JR, Holl MMB. 2006. Interaction of polycationic polymers with supported lipid bilayers and cells: Nanoscale hole formation and enhanced membrane permeability. Bioconjugate Chemistry 17(3):728-734.
Hussain N, Jaitley V, Florence AT. 2001. Recent advances in the understanding of uptake of microparticulates across the gastrointestinal lymphatics. Adv Drug Deliv Rev 50(1-2):107-42.
Jani P, Halbert GW, Langridge J, Florence AT. 1990. Nanoparticle uptake by the rat gastrointestinal mucosa: quantitation and particle size dependency. J Pharm Pharmacol 42(12):821-6.
Ji ZQ, Sun HF, Wang HF, Xie QY, Liu YF, Wang Z. 2006. Biodistribution and tumor uptake of C-60(OH)(x) in mice. Journal of Nanoparticle Research 8(1):53-63.
Jia X, Li N, Chen J. 2005. A subchronic toxicity study of elemental Nano-Se in Sprague-Dawley rats. Life Sci 76(17):1989-2003.
Kabanov AV. 2006. Polymer genomics: An insight into pharmacology and toxicology of nanomedicines. Advanced Drug Delivery Reviews 58(15):1597-1621.
Kampers F. 2007. Micro- and nanotechnologies for food and nutrition in preventative healthcare. . Food Science & Technology 21(1).
Kraehenbuhl JP, Neutra MR. 2000. Epithelial M cells: Differentiation and function. Annual Review of Cell and Developmental Biology 16:301-332.
Kroes R, Muller D, Lambe J, Lowik MRH, van Klaveren J, Kleiner J, Massey R, Mayer S, Urieta I, Verger P and others. 2002. Assessment of intake from the diet. Food and Chemical Toxicology 40(2-3):327-385.
Letchford K, Burt H. 2007. A review of the formation and classification of amphiphilic block copolymer nanoparticulate structures: micelles, nanospheres, nanocapsules and polymersomes. Eur J Pharm Biopharm 65(3):259-69.
Lomer MC, Hutchinson C, Volkert S, Greenfield SM, Catterall A, Thompson RP, Powell JJ. 2004. Dietary sources of inorganic microparticles and their intake in healthy subjects and patients with Crohn's disease. Br J Nutr 92(6):947-55.
Lomer MC, Thompson RP, Powell JJ. 2002. Fine and ultrafine particles of the diet: influence on the mucosal immune response and association with Crohn's disease. Proc Nutr Soc 61(1):123-30.
Luck M, Schroder W, Harnisch S, Thode K, Blunk T, Paulke BR, Kresse M, Muller RH. 1997. Identification of plasma proteins facilitated by enrichment on particulate surfaces: Analysis by two-dimensional electrophoresis and N-terminal microsequencing. Electrophoresis 18(15):2961-2967.
Maynard AD. 2006. Nanotechnology: assessing the risks. Nano Today 1(2):22-33.
Maynard AD, Aitken RJ, Butz T, Colvin V, Donaldson K, Oberdorster G, Philbert MA, Ryan J, Seaton A, Stone V and others. 2006. Safe handling of nanotechnology. Nature 444(7117):267-9.
Medina C, Santos-Martinez MJ, Radomski A, Corrigan OI, Radomski MW. 2007. Nanoparticles: pharmacological and toxicological significance. Br J Pharmacol 150(5):552-8.
Morgan K. 2005. Development of a preliminary framework for informing the risk analysis and risk management of nanoparticles. Risk Anal 25(6):1621-35.
Mozafari MR, Flanagan J, Matia-Merino L, Awati A, Omri A, Suntres ZE, Singh H. 2006. Recent trends in the lipid-based nanoencapsulation of antioxidants and their role in foods. Journal of the Science of Food and Agriculture 86(13):2038-2045.
Muller RH, Jacobs C, Kayser O. 2001. Nanosuspensions as particulate drug formulations in therapy. Rationale for development and what we can expect for the future. Adv Drug Deliv Rev 47(1):3-19.

Nacka F, Cansell M, Gouygou JP, Gerbeaud C, Meleard P, Entressangles B. 2001. Physical and chemical stability of marine lipid-based liposomes under acid conditions. Colloids and Surfaces B-Biointerfaces 20(3):257-266.
Nanoforum. 2006. Nanotechnology in agriculture and food.
Nel A, Xia T, Madler L, Li N. 2006. Toxic potential of materials at the nanolevel. Science 311(5761):622-7.
Nemmar A, Hoet PHM, Vanquickenborne B, Dinsdale D, Thomeer M, Hoylaerts MF, Vanbilloen H, Mortelmans L, Nemery B. 2002. Passage of inhaled particles into the blood circulation in humans. Circulation 105(4):411-414.
Oberdorster G, Maynard A, Donaldson K, Castranova V, Fitzpatrick J, Ausman K, Carter J, Karn B, Kreyling W, Lai D and others. 2005a. Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy. Part Fibre Toxicol 2:8.
Oberdorster G, Oberdorster E, Oberdorster J. 2005b. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ Health Perspect 113(7):823-39.
Oberdorster G, Utell MJ. 2002. Ultrafine particles in the urban air: to the respiratory tract--and beyond? Environ Health Perspect 110(8):A440-1.
Ogawara K, Yoshida M, Higaki K, Kimura T, Shiraishi K, Nishikawa M, Takakura Y, Hashida M. 1999. Hepatic uptake of polystyrene microspheres in rats: effect of particle size on intrahepatic distribution. J Control Release 59(1):15-22.
Ogawara K. YM, Furumoto K., Takakura Y., Hashida M., Higaki K., Kimura T. . 1999. Uptake by hepatocytes and biliary excretion of intravenously administered polystyrene microspheres in rats. J Drug Target 7(3):213-21.
Panyam J, Labhasetwar V. 2003. Biodegradable nanoparticles for drug and gene delivery to cells and tissue. Advanced Drug Delivery Reviews 55(3):329-347.
Powers KW, Brown SC, Krishna VB, Wasdo SC, Moudgil BM, Roberts SM. 2006. Research strategies for safety evaluation of nanomaterials. Part VI. Characterization of nanoscale particles for toxicological evaluation. Toxicol Sci 90(2):296-303.
Powers KW, M. P, Moudgil BM, Roberts SM. 2007. Characterization of the size , shape and state of dispersion of nanoparticles for toxicological studies. Nanotoxicology 1(1):42-51.
Preining O. 1998. The physical nature of very, very small particles and its impact on their behaviour. Journal of Aerosol Science 29(5-6):481-495.
Rajagopalan P, Wudl F, Schinazi RF, Boudinot FD. 1996. Pharmacokinetics of a water-soluble fullerene in rats. Antimicrobial Agents and Chemotherapy 40(10):2262-2265.
Roco MC. 2004. Nanoscale science and engineering: Unifying and transforming tools. Aiche Journal 50(5):890-897.
Rohner F, Ernst FO, Arnold M, Hilbe M, Biebinger R, Ehrensperger F, Pratsinis SE, Langhans W, Hurrell RF, Zimmermann MB. 2007. Synthesis, characterization, and bioavailability in rats of ferric phosphate nanoparticles. J Nutr 137(3):614-9.
Roszek B, de Jong W, Geertsma R. 2005. Nanotechnology in medical applications: State-of-the-art in materials and devices. . RIVM report 265001001/2005.
Rothen-Rutishauser BM, Schurch S, Haenni B, Kapp N, Gehr P. 2006. Interaction of fine particles and nanoparticles with red blood cells visualized with advanced microscopic techniques. Environ Sci Technol 40(14):4353-9.
Roy K, Mao HQ, Huang SK, Leong KW. 1999. Oral gene delivery with chitosan-DNA nanoparticles generates immunologic protection in a murine model of peanut allergy. Nature Medicine 5(4):387-391.
Russell-Jones GJ, Veitch H, Arthur L. 1999. Lectin-mediated transport of nanoparticles across Caco-2 and OK cells. Int J Pharm 190(2):165-74.
Salamat-Miller N, Johnston TP. 2005. Current strategies used to enhance the paracellular transport of therapeutic polypeptides across the intestinal epithelium. International Journal of Pharmaceutics 294(1-2):201-216.
Sayes CM, Fortner JD, Guo W, Lyon D, Boyd AM, Ausman KD, Tao YJ, Sitharaman B, Wilson LJ, Huges JB and others. 2004. The differential cytotoxicity of water-soluble fullerenes. . Nano Letters 4(10):1881-7.

SCENIHR. 2006. Scientific Committee on Emerging and Newly Identified Health Risk SCENIHR. Opinion on: The appropriateness of existing methodologies to assess the potential risk associated with engineered and adventitious products of nanotechnologies. European Commision Health & Consumer Protection Directorate-General. Directorate C - Public Health and Risk Assesment C7- Risk Assessment. .

SCENIHR. 2007. Scientific Committee on Emerging and Newly Identified Health Risk SCENIHR. Opinion on: The appropriateness of the risk assessment methodology in accordanxe with the technical guidance documents for new ans existing substances for assessing the risks of nanomaterials European Commision Health & Consumer Protection Directorate-General. Directorate C - Public Health and Risk Assesment C7- Risk Assessment.

Silva GA. 2007. Nanotechnology approaches for drug and small molecule delivery across the blood brain barrier. Surgical Neurology 67(2):113-116.

Singh R, Pantarotto D, Lacerda L, Pastorin G, Klumpp C, Prato M, Bianco A, Kostarelos K. 2006. Tissue biodistribution and blood clearance rates of intravenously administered carbon nanotube radiotracers. Proc Natl Acad Sci U S A 103(9):3357-62.

Taylor TM, Davidson PM, Bruce BD, Weiss J. 2005. Liposomal nanocapsules in food science and agriculture. Crit Rev Food Sci Nutr 45(7-8):587-605.

Thomas K, Sayre P. 2005. Research strategies for safety evaluation of nanomaterials, Part I: evaluating the human health implications of exposure to nanoscale materials. Toxicol Sci 87(2):316-21.

Tran CL, Danaldson K, Stones V, Fernandez T, Ford A, Christofi N, Ayres JG, Steiner M, Hurley JF, Aitken RJ and others. 2005. A scoping study to identify hazard data needs for addressing risks presented by nanoparticles and nanotubes. . Institute of Occupational Medicine (IOM) Research Report, Edinburgh, United Kingdom. .

Tsuchiya T, Oguri I, Yamakoshi YN, Miyata N. 1996. Novel harmful effects of [60]fullerene on mouse embryos in vitro and in vivo. FEBS Lett 393(1):139-45.

Ulrich AS. 2002. Biophysical aspects of using liposomes as delivery vehicles. Biosci Rep 22(2):129-50.

Wang B, Feng WY, Wang TC, Jia G, Wang M, Shi JW, Zhang F, Zhao YL, Chai ZF. 2006. Acute toxicity of nano- and micro-scale zinc powder in healthy adult mice. Toxicol Lett 161(2):115-23.

Wang J, Zhou G, Chen C, Yu H, Wang T, Ma Y, Jia G, Gao Y, Li B, Sun J and others. 2007. Acute toxicity and biodistribution of different sized titanium dioxide particles in mice after oral administration. Toxicol Lett 168(2):176-85.

Weiss J, Takhistov P, McClements J. 2006. Functional materials in food nanotechnology. Journal of Food Science 71(9):R107-R116.

Xia Z, Wang, G, Tao, K, Li, J, Tian, Y. 2005. Preparation and acute toxicology of nano-magnetic ferrofluid. . J-Huazhong-Univ-Sci-Technolog-Med-Sci 25 (1):59-61 (abstract).

Zhang J, Wang H, Yan X, Zhang L. 2005. Comparison of short-term toxicity between Nano-Se and selenite in mice. Life Sci 76(10):1099-109.

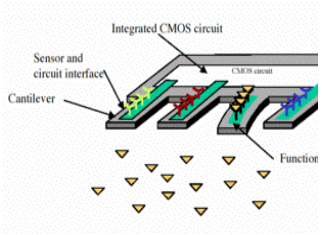
Zhang JS, Gao XY, Zhang LD, Bao YP. 2001. Biological effects of a nano red elemental selenium. Biofactors 15(1):27-38.

Annex 1 Inventory of applications of nanotechnol[4]ogy in the food chain

6.1.1 Nanosensors

Nanotechnology applied in agriculture. Terminology like precision farming is used, meaning that autonomous nanosensors are applied for real-time monitoring and early identification of plant health issues to take appropriate measures as early as possible. Use in early warning or emerging risk approaches can be foreseen.

Sensors (including radio frequency identification technology) can also be incorporated into packaging materials (see packaging materials). Or sprayed directly on commodities.

Product	Basic element	Function	Illustration	Other information	Reference
NanoBiolumines cence Detection Spray	Contains luminescent proteins that bind to the surface of Salmonella and E. coli	When bound it emits a visible glow. In addition spray techniques are developed to apply these sensors in ocean freight containerized shipping			http://www.agromicron.com /BTP.htm
BioFinger	Nano and micro cantilevers coated with antibodies	Versatile, inexpensive, and easy-to-use diagnostic tools for health, enviroNPental and other applications		In final developmental stage. Analysis based on the measurement of molecular interactions (ligand-receptor interactions) by integrated micro- and nano-cantilever sensors. These are based on static and resonant cantilever arrays, which contain surface-stress or mass-sensitive elements, respectively.	http://www.iqe.ethz.ch/pel/r esearch/biofinger.html

[4] Pictures in all tables of annex 1 are taken from the website that is referred to in the column reference of this table

6.1.2 Pesticides (delivery systems)

Nanoformulations of pesticides are marketed and developed. In general the aim is to increase solubility in water, increase efficacy. Research is ongoing to develop encapsulates that are response to changes in the enviroNPent: e.g. humidity, pH etc.

Product	Basic element	Form	Size	Function	Other information	Reference
Nanoemulsion Primo MAXX®	Trinexapac-ethyl(cimectacarb)	micoremulsionconcentrate	100NP	mix completely with water and not settle out in a spray tank	Registered for use in USA (some states)	Syngenta http://www.syngentaprofessionalproducts.com/to/prod/primo/
Controlled release Karate®ZEON	lambda-cyhalothrin	Quick-Release Encapsulation	2.5 micron	Quick release. Improve residual function. Protection from UV	Registered for use in USA, Germany, Brazil, France, India, Mexico, Indonesia, United Kingdom, Canada	Syngenta http://www.syngentacropprotection-us.com/prod/insecticide/Karate/
Controlled release Demand® CS	lambda-cyhalothrin	Encapsulation	2.5 micron		Also for indoor use	Syngenta http://www.syngentaprofessionalproducts.com/ppm/prod/demand/
Iconet™	lambda-cyhalothrin	Encapsulation	2.5 micron		Bednets repellent (Malaria)	Syngenta http://www.syngenta.com/en/products_services/icon_page.aspx
"gutbuster"	Various active ingredients	--	--	research on triggered-release capsules whose outer shell can be opened only in special conditions.	R&D stage	Syngenta http://www.syngenta.com/en/day_in_life/microcaps.aspx

6.1.3 Water purification/ soil cleaning

Purification of water which may be used in food production can involve filtering through nanofilters and purification due to reaction of contaminants with nano-sized metal particles. The latter method can in return be separated in catalysation or binding. The bioavailablity of the particles after binding is unknown.

Product	Basic element	Form	Size	Charge	Function	Illustration	Other information	Reference
Nanofiltration	--	--	--	--	Filtering		Not known	Generale des Eax + Filmtec www.nanoforum.org
Nanofiltration NanoCeram	Aluminium oxide	2 NP AlO nanofibers	--	--	Filtering			Argonide http://www.argonide.com/index.htm
Water cleaning Colloid	Lanthanum particles	--	--	--	Removing phosphates and arsenate			Altairno http://www.altairnano.com
Soil cleaning	Nanoscale iron powder	--	--	--	Catalysing oxidation organic contaminants			Lehigh Universisty www.nanoforum.org
Water cleaning (colloid)	Nanoscale iron powder	--	--	--	Binding and removing arsenic			Centre for Biological and EnviroNPental Nanotechnology www.nanoforum.org

Filter systems and other purification techniques are being developed by many companies (e.g. BASF, DowChemicals)

6.1.4 *Food processing and storage*

Mostly silver is used in processing/storage materials for its anti-bacterial properties, with exception of Zinc oxide in plastic wrapping. For Oil Fresh, the nano-element is unknown.

Product	Basic element	Form	Size	Charge	Function	Illustration	Other information	Reference
Nanoceramic inserts for deepfryers	Unknown				Catalytically inhibiting thermal polymerization (molecules lumping together) process of the frying oil		A flat, semi-permanent, vertical insert, made of an advanced nanoceramic material. Authorized by the US FDA and certified by NSF Int'l.	Oil fresh http://www.oilfresh.com/
Antibacterial kitchen- and table ware	Silver				Anti-bacterial			Nano Care technology http://www.nanocaretech.com/enyeNewsInfo.asp?id=17
Silver nano baby bottle and baby mug cup	Silver						Through silver nano poly system 99.9% of germs are prevented and it maintains anti-bacteria, deodorizing function as well as freshness.	Baby dream http://babydream.en.ec21.com/
Fresher longer Miracle food storage containers and bags	Silver				Anti-bacterial		Patent-pending silicone gasket locking system keeps out oxidizing air and antimicrobial silver nanoparticles in the polypropylene material reduce the growth of microorganisms.	Sharper Image http://www.sharperimage.com/us/en/catalog/product/sku__ZN020
Nano plastic wrap	ZnO				Anti-UV, reflecting IR, sterilizing and anti-mould, better temparture resistance, fire proof, bearing grinding			SongSing Nano Technology http://www.ssnano.net/ehtml/detail1.php?productid=79

Nano silver tea pot	Silver	Anti-bacterial, getting rid of bitter tea taste		SongSing Nano Technology http://www.ssnano.net/ehtml/detail1.php?productid=73
Nano silver spray	Silver	Sterilization and deodorization		http://www.ssnano.net/ehtml/detail1.php?productid=73
Food Container (NS)	Silver	Anti-bacterial		A-Do Global http://www.adox.info/?doc=shop/list.php&ca_id=110
Nano Silver Cutting Board	Silver	Anti-bacterial		A-Do Global http://www.adox.info/?doc=shop/item.php&it_id=000123
Silver coatings in refrigerators	Silver			LG, Samsung, Daewoo

6.1.5 Food packaging

Smart packaging methods are developed to optimize shelf-life and improve food safety. These systems would be able to repair small holes, respond to enviroNPental conditions and alert the customer if the food is contaminated or spoiled. NPs included in plastic can increase barrier properties, and used to develop active antimicrobial and antifungal surfaces (www.nanoforum.org).

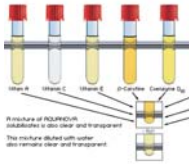
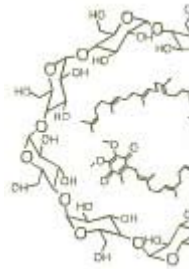
Product	Basic element	Form	Size	Charge	Function	Illustration	Other information	Reference
" electronic tongue"	Nanosensor				Sensitve to gasses (spoiling of foods)			Kraft Foods a.o. www.nanoforum.org
NanoBiolumines cence Detection Spray					When bound it emits a visible glow. In addition spray techniques are developed to apply these sensors in ocean freight containerized shipping			
Durethan KU2-2601	Silicate NPs				Packaging film, barrier properties			BASF www.nanoforum.or g
"Aegis" nylon 6	Nanocompos ite				Increased barrier properties			Honeywell Speciality www.nanoforum.org
Anitmicrobial films					Absorbs oxygen from the content of the package			Kodak www.nanoforum.org
Imperm	Nanocompos ite containing clay NPs				Stonger bottles (for beer)			Voridan, Nanocor www.nanoforum.org
Dirt repellent coatings	e.g. Magnesium oxide Zinc oxide				'Lotus' effect Antimicrobial			www.nanoforum.org

6.1.6 *Food commodities: Inert particles*

Product	Basic element	Form	Size	Charge	Function	Illustration	Other information	Reference
Nano Cal/Mag	Calcium and magnesium	Unknown	Unknown	Unknown	Enhanced uptake supplements		From plant origen.	http://www.mag-i-cal.com/calciummagnesium.htm#nanocalmag
Silver 22	Silver	Colloid	Unknown	Unknown	Purifying and conservation of unknown targets.		Solved in water, at concentration of 22 ppm	http://www.rbclifesciences.com/Products.aspx?ItemID=430
Nanoceuticals Microhydrin	Silicate	Colloid	Unknown	Donates electrons	Anti-oxidant and reducing surface tension of water		Antioxidant by donating electrons and reducing surface tension of water from 73 to 45 dynes	http://www.rbclifesciences.com/Products.aspx?ItemID=161
Nanosilicea kapseln	Siliciumdioxide, magnesium and calcium	Colloid	Unknown	Unknown	Supporting cell structure and stability, and supporting physical condition.		-	http://www.neosino.com/index.php?id=695
Sovereign Silver	Silver	Colloid	Diameter 0.8 NP	Unknown	Supporting immune system		Concentration silver of 10 ppm	http://www.natural-immunogenics.com/silver_why_sovereign.php

Utopia silver supplements advanced colloidal silver	Silver	Colloid	Unknown	Unknown	Supporting immune system		Concentration silver of 20 ppm	http://www.utopiasilver.com/products/silver/
ASAP solutions	Silver	Colloid	Unknown	Unknown	Supporting immune system		10 ppm Producer claims in vitro and in vivo testing of product.	http://www.nanoshop.com/listing/321/Engineered_silver_nanoparticle_mineral_supplement.html http://www.asapsolution.com/
Shetec Platinum water	White gold	Colloid	2 NP	Unknown	Anti-oxidant by reacting with free radicals.			Print GNDP database
Biodream colloid plus	Silver	Colloid	5-15 NP	Yes, but charge unknown	Helpful against 'several ilnesses'	Unknown	Unknown	http://www.biodreamshop.nl/UserFiles/File/Colloid_A5_compleet.pdf

6.1.7 *Food commodities: Carriers*

Product	Basic element	Form	Size	Charge	Function	Illustration	Other information	Reference
Aquanova® Novasol®	Micell		Diameter 30 NP				100% water soluble, for solving normally insolvable substances.	http://www.nanotechproject.org/index.php?id=44&action=view&product_id=1194
Canola active oil	Micell		Unknown		Loaded with phytosterols		Replaces cholesterol in bile acid micells, preventing uptake in the blood.	http://www.nanotechproject.org/index.php?id=44&action=view&product_id=1019
LifePak Nano	CR6-liponutrients		Unknown		Loaded with anti-oxidants, vitamins and minerals		Supplement 'on demand', anything can be incorporated in the CR6 membrane.	http://www.pharmanex.com/intercom/productDetail.do?prodId=01003610&mktId=2031
Nanoceuticals Chocolate Slim Shake	Nanoclusters (contents unknown)		Unknown		Enhancing cocoa flavour and uptake of supplemented soy and whey proteins.		According to the producer, nanoclusters are tiny particles, 100,000th the size of a single grain of sand, and are designed to carry nutrition into your cells.	http://www.rbclifesciences.com/Products.aspx?ItemID=38

Nutralease nano-sized self-assembled liquid structures (NSSL) supplements	Micell	30 NP	Delivery system		Coined fortified nano-vehicles.	http://www.nutralease.com/technology.asp,
Solgar Nutri Nano Co Q10	Micell	30 NP	Enhanced uptake of Coenzym Q10		-	http://www.nanoshop.com/listing/59/Nano_nutritional_supplements.html
Tip Top Bread	Micell	Unknown	Uptake of tuna oil with omega 3 fatty acids without tastin git.		-	http://www.tiptop.com.au/driver.asp?page=main/products/bread/up+wholemeal+omega+3+dha
cheese	liposome		Entrapment of proteolitic enzymes in cheese produktion			(Mozafari et al. 2006)
	liposomes		Facilating intracellular uptake and extending the half live of encapsulated antioxidants			(Mozafari et al. 2006)

	Archaeosomes			Protect antioxidants during food processing and show great promise as an oral delivery system for bioactive agents.	Relatively thermostable, resistant to oxidation and enzymatic hydrolysis, low pH and bilesalts.	(Mozafari et al. 2006)
	Nanocochleates	Cigar shaped		Delivery system for hydrophobic, amphiphylic, neg. or pos charged molecules and resistant in the gastrointestinal tract		(Mozafari et al. 2006)
	Liposomes			Encapsulation of □-tocopherol	The disappearance rate after oral dose of 5,0000 U to cows of □-tocopherol was less when encapsulated in liposomes bud not significant.	(Bontempo et al. 2000)
	Liposomes Based on a natural mixture of marine lipids			Delivery of pufa by the oral route	Marine lipids constituted an attractive material for the development of liposomes	(Nacka et al. 2001)
	chitosan	sperical	100-200 nm	Carrier in oral allergen-gene immunization to treat food allergy		(Roy et al. 1999)
cheese	liposomes			Entrapment of encapsulated proteinases and lipases during cheese ripening		(Taylor et al. 2005)
Dairy products	liposomes			Fortify products with vitamins as well as aid in digestion and protection against degradation		(Taylor et al. 2005)

	Liposomes of marine phospholipids			Oral PUFA supplement		(Cansell et al. 2003)
	Polystyrene	spheres	50nm-3□m		Daily dose of 1.25 mg by oral gavage to rats 50 and 100 nm particles were absorbed and found in blood	Halbert 1990 Reviewed by (Hoet et al. 2004)

6.1.8 *Food commodities: Other applications*

Product	Basic element	Size	Function	Illustration	Other information	Reference
ASN advanced sports nutrition supplements	Nanosized particle	Under 1 µm	Advanced absorption		Nanosized creatine particles, isolated from micronized creatinine.	http://www.asn-nutrition.com/nano_technology.htm
C.L.E.A.N. products (1-8)	Regulatory peptides from plants	0.01 – 0.1 NP	Supporting several body functions		To be solved in water.	https://www.sportmedix.com/index.php?lang=english&page=products&dlei_pp=1
Nanotechspray (oral dosing)	Nanodroplets	87 NP	Enhanced uptake of vitamin B12 and other supplements		Nanodroplets are produced by nanotechnology in the spraying system.	http://www.nutritionbynanotech.com/product_line.htm
Artichoke nanoclusters, Spirulina nanoclusters	Nanoclusters	Unknown	Supporting liver function, improvement of skin and support of total condition.		RBC lifsciences produces several types of NanoCeuticals. The 'nanoclusters' consist of potassium citrate, potassium carbonate, silica, purified water, magnesium sulfate and sunflower oil.	http://www.rbclifesciences.com/Products.aspx?ItemID=118

Shenzhen Nanotea	Nanomilled tea	<100NP	Enhanced uptake of tea ingredients.		Nanomilled tea.	http://www.nanotechproject.org/index.php?id=44&action=view&product_id=1228 www.369.com.cn/En/nanotea.htm https://www.uknow.or.jp/be/science/seminar/nanotech_business/simon_holland.pdf
Spray for life Vitamin supplements	Nanodroplets	0.188 NP and 5.421µm?	Enhanced uptake		According to producer, Nanodroplets™ are made by a patented nanosuspension process, which allows molecules to be embedded into micro and Nanodroplets™ at an average of 0.188 NP and 5.421µm in size, which are used to create stable, uniform and highly soluble emulsions and dispersions.	http://www.healthplusintl.com/products.html
Nanoscale coating	Variable	Unknown	Accurate coating of food products		Ultrasonic spraying systems are used to accurately put natural anti-bacterial coats on food products. The process was also used with natural oils and various glazing and decorating compounds.	http://www.foodqualitynews.com/news/ng.asp?n=76650&m=2FQN518&c=gqcohokhydnruud http://www.sono-tek.com/widetrack/index.php
Nanoceuticals Hydracell	Nanoclusters	Unknown	Reducing surface tension of water.		Reduces the surface tension of water from 74 dynes to 59 dynes. The mechanism is not mentioned.	http://www.rbclifesciences.com/Products.aspx?ItemID=142

Nano Water	Waterclusters	Unknown	Enhanced uptake of water		According to producer, Nanowater is processed by the Nanometer high-energy water activator which enhances the activity of water molecule clusters and shrinks the molecule clusters so that the infiltration pressure is strengthened and that the speed of movement of the small water molecule clusters is increased.	http://www.nanotech.com.hk/en/first.html

Annex 2: Summaries of available oral toxicity studies

(Chen et al. 1998)studied the acute and subacute toxicity of C60 polyalkylsulfonate in rats. No mortality was observed in an acute oral toxicity test with doses up to 2500 mg/kg. After ingestion, NPs may cross the gut barrier and can be distributed to various organs depending on their size. There is insufficient evidence to determine whether NPs adversely affect the gut or the organs they are distributed to (Tran et al., 2005; UBA, 2006).

(Wang et al. 2006)studied the acute toxicity of oral exposure to nanoscale zinc powder compared to microscale zinc powder in mice. The mice were gastrointestinally administered at a dose of 5 g/kg body weight. The nanoscale zinc treated mice showed more severe symptoms of lethargy, vomiting and diarrhea in the beginning days than the microscale zinc treated mice. Deaths of two (of the ten) mice occurred in the nanoscale zinc group after the first week of treatment due to intestinal obstruction of the nanoscale zinc aggregation. Clinical changes and biochemical liver function tests of serum indicated that microscale zinc powder induced more severe liver damage than nanoscale zinc powder. Histopathological examinations showed severe renal damage in the nanoscale zinc treated mice, without significant changes of blood biochemical levels. Blood-element test indicated that nanoscale zinc powder could cause severe anemia. Besides the pathological lesions in the liver, renal, and heart tissue, only slight stomach and intestinal inflammation was found in all the zinc treated mice, without significant pathological changes in other organs.

The toxicity of copper NPs (23.5 NP) exposed to mice by oral gavage was compared with that of copper micro-particles (17μm) and cupric ions (CuCl2·2H2O) (Chen et al., 2006). The LD50s of 23.5 NP, 17 μm copper particles and cupric ions were determined to be 413, >5000 and 110 mg/kg body weight, respectively. The pathological examinations revealed that kidney, liver and spleen are target organs for nano-copper particles. These were further demonstrated by measurements of the blood biochemical indexes (BUN, Cr, TBA and ALP) reflecting the renal and hepatic functions of experimental mice. Pathological changes and grave injuries on kidney, liver and spleen were observed in mice exposed to 23.5 NP nano-copper particles (e.g., swelling up and dwindling in gap of renal glomerulus, degeneration and irreversibly massive necrobiosis of epithelial cells of renal proximal convoluted tubules, reducing karyons of epithelial cells of renal tubules, proteinic liquid in renal tubules, purple deposition in the proteinic liquid, the steatosis around venae centrals of hepatic tissue, etc.), but they were not found in mice exposed to 17 μm copper particles on mass basis. In addition, toxicity of nanocopper is sex-dependent: male mice exhibit more severe toxic symptoms and suffer more from nanocopper than females after they exposed to the same mass of particles.
Wang et al. (2007) investigated the acute oral toxicity of 25, 80 NP and fine (155 NP) TiO2 particles was investigated according to the standard procedure (OECD Guidelines, No. 420) for testing the chemicals. No obvious acute toxicity was observed after a single oral exposure to 5 g/kg TiO2 particles. However, the female mice showed higher coefficients of liver in the nano-sized (25 and 80 NP) groups than the fine group. The changes of serum biochemical parameters (ALT/AST, LDH) and pathology (hydropic degeneration around the central vein and the spotty necrosis of hepatocytes) of liver indicated that the hepatic injury was induced after exposure to mass different-sized TiO2 particles. In addition, the nephrotoxicity like increased BUN level and pathology change of kidneys was also observed in the experimental groups. The significant change of serum LDH and alpha-HBDH in 25 and 80 NP groups showed the myocardial damage compared with the control group. However, there are no abnormal pathology changes in the heart, lung, testicle (ovary), and spleen tissues. Biodistribution experiment showed that TiO2 mainly retained in the liver, spleen, kidneys, and lung tissues, which indicated that TiO2 particles could be transported to other tissues and organs after uptake by gastrointestinal tract.

(Zhang et al. 2001)showed that Nano-Se had lower acute toxicity as compared with selenite in mice (LD50: 113.0 vs. 15.7 mg/kg bw Se). However the bioavailability of selenite was similar in terms of inducing seleno-enzymes in cultured cells and in Se-deficient rats.
Zhang et al., (2005) compared the short-term toxicity of both selenite and Nano-Se in mice. An oral dose of 2, 4 and 6 mg selenite or Nano-Se /kg bw per day was administered for consecutive 12 or 15 days. Nano-Se is less toxic than selenite in short-term/large dose treatments as shown by ameliorated suppression of growth, moderate redox stress, and liver toxicity (lower levels of ALT and AST).

(Jia et al. 2005)compared the subchronic toxicity of Nano-Se with selenite and high-selenium protein in rats. Groups of Sprague-Dawley rats (12 males and 12 females per group) were fed diets containing Nano-Se, selenite and highselenium protein at concentrations of 0, 2, 3, 4 and 5 ppm Se, respectively, for 13 weeks. At the two higher doses (4 and 5 ppm Se), significant abnormal changes were found in body weight, hematology, clinical chemistry, relative organ weights and histopathology parameters. However, the toxicity was more pronounced in the selenite and high-selenium protein groups than the Nano-Se group. At the dose of 3 ppm Se, significant growth inhibition and degeneration of liver cells were found in the selenite and high-selenium protein groups, but not in the Nano-Se group. In conclusion, Nano-Se is less toxic than selenite and high-selenium protein in the 13-week rat study.

In studies with cationic PAMAM dendrimers, Duncan et al. ((Duncan and Izzo 2005)) administered generations 3, 5 and 7 to mice at doses of 2.6, 10 and 45 mg/kg, respectively. The dendrimers were given either as single dose or repeatedly once a week for 10 weeks. Although no behavioural changes or weight loss was reported over a 2 h period, after administration of generation 7 three animals died. In the multiple dose study a degree of liver cell vacuolation was also observed during histopathology and this would be consistent with a lysosomal storage problem. Further studies are needed to verify these findings (Duncan et al., 2005).
Carrero-Sanchez et al., (2006) compared the toxicological effects between pure carbon multiwalled nanotubes (MWNTs) and N-doped multiwalled carbon (CNx) nanotubes. Different doses of tubes were administered in various ways to mice: nasal, oral, intratracheal, and intraperitoneal. When MWNTs were injected into the mice's trachea, the mice could die by dyspnea depending on the MWNTs doses. However, CNx nanotubes never caused the death of any mouse. CNx nanotubes were far more tolerated by the mice when compared to MWNTs. Extremely high concentrations of CNx nanotubes administrated directly into the mice's trachea only induced granulomatous inflammatory responses. Importantly, all other routes of administration did not induce signs of distress or tissue changes on any treated mouse. These results indicate that CNx nanotubes are less harmful than MWNTs or SWNTs.

(Xia 2005)studied the acute toxicology of nano-magnetic ferrofluid. The effective diameter of the magnetic particles was about 19.9 NP, and the concentration of the ferrofluid was 17. 54 mg/ml. The acute toxic reaction and the main viscera pathological morphology of mice were evaluated after oral, intravenous and intraperitoneal administration of the nano-magnetic ferrofluid of different doses respectively. Half lethal dose (LD50) > 2104. 8 mg/kg, maximum non-effect dose (ED0) = 320. 10mg/kg with oral; LDs,> 438. 50 mg/kg, EDo = 160. 05 mg/kg with intravenous route; and LDso >1578. 6 mg/kg, ED0 = 320. 10 mg/kg with intraperitoneal administration. Degeneration and necrosis of viscera were not found. These results indicate that the acute toxicity of nano-magnetic ferrofluid is very low.