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Discussion

The choreography of chemicals in nature; beyond ecotoxicological limits

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HIGHLIGHTS

- Chemicals are in every compartment and have a dynamic choreography.
- As humans, only rarely we foresee the consequences of our actions.
- Who is the choreographer and what is his/her story.
- Look beyond the ecotoxicological limits and account fringing ecosystems.
- Risk Assessment and Life Cycle Analysis needed to guide sustainable technologies.

GRAPHICAL ABSTRACT



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1. Chemicals in nature

“To Respect the Earth’s Limits — or Push Them?” that are the two outspoken stances within the today’s environmental debates, and how the book written by Mann (2018), “The Wizard and the Prophet”, is summarized. The Wizard is the technocrat, embodied

by Borlaug the Nobel-winning Midwest agronomist who stood on the basis of the Green (agricultural) Revolution. Vogt was the environmentalism with its sense that humans should respect natural limits — he is the “Prophet”. Currently there is a wave of awareness (e.g. Raworth, 2018; Rockström et al., 2009) that humankind is pushing the planetary boundaries by their innovations in products and new chemicals. As an ecotoxicologist I focus on what happens when chemicals find their way into nature. *Where* do they go, *how many* are there and do they pose a *threat* to the ecosystem? My discussion paper is intended to give insights in the scientific debates that are held within the environmental studies and more explicitly into the scientific field of environmental toxicology embedded in the world that is struggling with sustainability. It has been written based on my inaugural speech, and explicitly written in such a way that it is accessible for those scientists that are broadly interested related to all aspects of environmental science and engineering. To illustrate the theories, results of different type of research is given.

The substances we humans synthesise and extract are used in myriad ways: in products in industry, in agriculture, in our homes. We introduce these substances into the system, but that only goes well if they stay exactly where we intend, in just the right quantity, within what we might call system boundaries — a term I freely adapt from the work of Daly (2000), and as also done by Rockström et al. (2009) and Raworth (2018). Unintentionally, the substances end up in nature at large, though, where their concentration may

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become so high as to exceed environmental quality standards, posing a hazard to the plants and animals out there – at which point they become “problematic substances”. We know from data reported in our online Pesticides Atlas that water quality standards are exceeded at up to 50% of monitoring sites in the Netherlands (www.bestrijdingsmiddelenatlas.nl). This is undesirable, but how significant is it – does it count as pollution? Anthropologist Mary Douglas has thought about this. In her 1966 book *Purity and Danger* she identifies concerns about purity as a key theme at the heart of every society. Her book has been highly influential in shaping thought from religious studies and social theory through to environmental science. Her key insight was to equate “pollution” with “matter out of place”, that is, *in the wrong place*. From the perspective of ecotoxicology we are then talking mainly about substances that are problematic in light of their poor degradability, high toxicity or tendency to accumulate, as well as about just *too many* substances.

It is important to realise that in our industrialised world there are an incredible number of substances that can potentially lead to pollution, to “matter out of place”. Since 1950 we have synthesised over 140,000 chemicals, including pesticides, the vast majority of which are still in production today. To give you an idea of the scale: to build a car and keep it up and running for its allotted life span requires over 10,000 different compounds. If we are serious about assessing *risks* to the ecosystem, we need to know something about *all* of them. To collect data and build up a body of knowledge on these issues, two methods are conventionally used: field monitoring and laboratory trials. Data gathered by monitoring tells us something about the chemical or biological quality of the real world. If the monitoring is carried out at repeated intervals, statistics can be used to build up a spatiotemporal picture of the state of the ecosystem. Gathering data in this way is by definition a retrospective approach, as the substances must already be present in nature for us to measure them. The laboratory testing (see Fig. 1A) – in tandem with predictive models – can provide insight into what kinds of impacts are to be expected in nature. In this case, then, we have a prospective approach.

The key question is; can we provide and use products that come with synthesised chemicals without wrecking much else? The focus of my own studies is on making realistic predictions about *how* substances can potentially affect our natural environment and the organisms to which it is home. This notorious challenge I am happy to take on, guided by the notion of “matter out of place” proposed by Mary Douglas. In doing so, I see the movement of substances through the environment as a dance, as a choreography. In this discussion paper I shall be exploring two storylines: first the choreography of crop protection agents, then that of nanomaterials. Together, these storylines illustrate the issues of interest in the ecotoxicology discipline and point to some of the traps and challenges encountered.

2. The dance of the agrochemicals

The first choreography concerns agrochemicals. As a case study we follow on neonicotinoids, a class of compounds that are among the world's most widely used insecticides. They are the latest replacement of the organophosphates of yesteryear, with a specific mode of action and relatively swift biodegradability compared with those earlier compounds. Thanks to their systemic action, neonicotinoids are an effective means of controlling insect damage. One advantage of this form of pest control is that the crop no longer needs to be sprayed, thus avoiding undesirable drift to adjacent surface waters and other habitats. Before these chemicals were approved they underwent extensive testing to determine their physicochemical properties and degradability and their toxicity

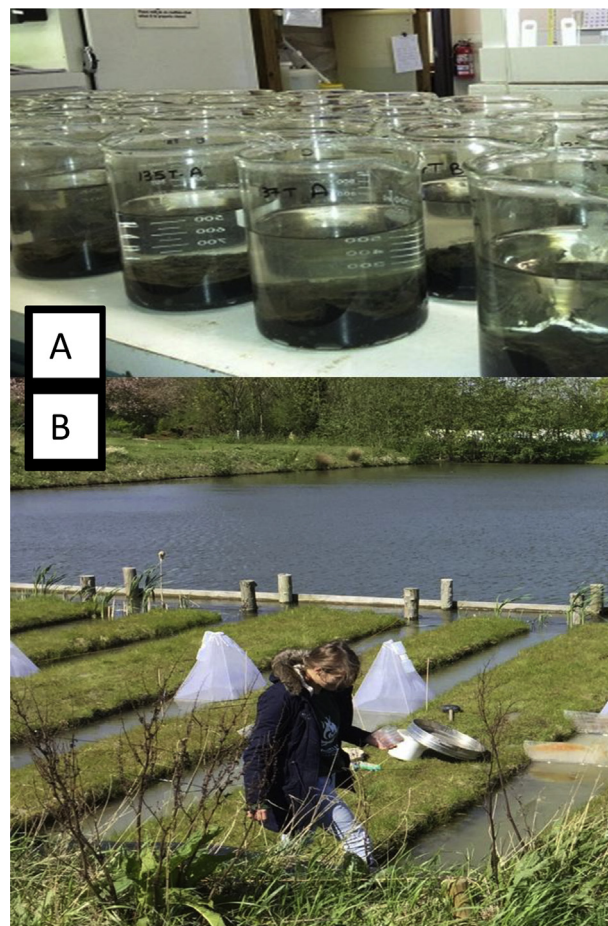


Fig. 1. Different aquatic ecotoxicological testing. A. indoor experiment (example microcosm water-sediment interface). These tests are performed under standardized conditions with fixed temperature and day/night regime, single organisms that are easy-to-culture and ad libitum food provided every two days. B. outdoor experiment (example experimental ditches at facility Leend Lab, Universiteit Leiden). These tests are performed under natural conditions with fluctuating weather conditions, community or food web levels, indigenous organisms with biotic interactions such as food web and community and abiotic interactions.

was assessed in the laboratory using a number of standard species. Studies by the U.S. Environmental Protection Agency have shown that many insect and invertebrates species respond to neonicotinoids, but with major interspecies differences in sensitivity. It has also been established that other species may potentially be impacted. Amphibians and fishes appear to be insensitive to imidacloprid. Water fleas – small crustaceans used as the standard organism in many toxicity tests – exhibit average sensitivity.

In 2013 Van Dijk and co-authors reported, on the basis of field observations in the Netherlands, a negative correlation between surface-water concentrations of imidacloprid and populations of various classes of aquatic invertebrate. Although we (Vijver and van den Brink, 2014) have some issues with how the study was conducted, we too conclude, following other lines of evidence, that current Dutch imidacloprid levels are most definitely impacting aquatic organisms. A report by the European Academies of Science (European Academies Science Advisory Council, 2015) then showed that honeybees (as well as multiple species of wild bees, bumblebees, butterflies and hoverflies) are extremely sensitive to very low doses of neonicotinoids. The decline of fifteen bird species in rural areas also correlates with surface-water imidacloprid levels (Hallmann et al., 2014), as does the sharp decline in insect numbers

(Hallmann et al. 2018), and aquatic organisms were also found to be impacted more than anticipated by neonicotinoid-coated seeds (Vijver et al., 2017). Using field data Ieromina et al. (2016) showed that biotic interactions of species abundant in ditches adjacent to agricultural fields where insecticides were used are consequently a crucial element in explaining how a system responds to chemical pollution. What this has taught us is that pollutants need to be studied as a “syndrome”, not isolated from their context of biotic and abiotic interactions. The moral of this story – this dance – is that lab results underestimate pollution impacts for a great many organisms found in the ecosystems bordering on farmland and greenhouses. In general only 1 in 10 “problems” are registered in the laboratory, even though lab analyses are our key means of proactively addressing adverse impacts. Within the EU project SOLUTIONS (<https://www.solutions-project.eu/>) effective tools of monitorings approaches and bio- and chemo-analytical tools are developed. But next to new tools, what we need is a scientifically sound means of translating from laboratory to field.

Ecosystem complexity as a whole is needed to be incorporated enabling to understand chemical-induced impacts. This new integration of knowledge requires a experimenting under semi-realistic field conditions. The idea of “mesocosm studies” goes back to the 1990s (with OECD guidelines dating from 2004). Mesocosms are small artificial ecosystems that can provide data on overall system functioning. As in the cosms, this kind of set-up can be manipulated and test protocols developed. Its strength lies in it having the replicability of the lab, but involving outside facilities maintained under natural conditions. An example can be found in our Levend Lab (www.mesocosms.eu), see Fig. 1B.

First, because we are not just looking at a single test species of animal or plant, but at complete communities as are to be found in adjacent nature. The organisms come straight out of the surrounding water courses via the lake and the ditches are bounded by natural banks. Second, our experiments go on for an extended period, of key importance because human influence is often only apparent over the longer term, while lab tests are generally very short-term. Our experiments are monitored all year round.

The results obtained with our mesocosm experiments show that when two different agrochemicals are added to the system – a binary mixture – the toxicity is predicted well by our lab-based knowledge. When more than two compounds are added simultaneously, though, the toxicity can no longer be forecast from lab results (Barmiento et al., 2018a). When we shifted our research focus to chemicals in combination with food availability, Barmiento et al. (2018b) saw that caged water fleas put out in ditches to which thiacloprid had been added were an incredible 2500 times more sensitive under field conditions than in the lab. We also investigated the more complex inter-species interactions. Here we discovered similar, unexpected effects. We found, for example, that adding thiacloprid at environmentally relevant levels led to a shift in community composition. One thing that was plainly visible after two months was the enormous quantity of algae that had developed, even though this was essentially a nutrient-poor ecosystem. What was driving this algal bloom – a phenomenon normally associated with eutrophication – was the fact that the herbivores had disappeared. There was a direct impact on food chains, with inter-species relationships being disturbed (Schrama et al., 2017), and these can have consequences even across ecosystem boundaries. An example is the how alterations of natural light/dark cycles caused by artificial light at night have been shown to exert reproductive and behavioural effects (Navara and Nelson, 2007) and alters migration and feeding (Perkin et al. 2011) as well as local freshwater insect emergence (Manfrin et al., 2018). This tells us the impact of pollution goes beyond simply affecting the chemistry and ecology of the water, then, all too evidently resonating across the

boundaries of the aquatic system into the biotic communities of land and air. Therefore the so-called cross-ecosystem effects need to be explicitly investigated, not only because pollution cross system boundaries, but also because ecological impacts echo into other, connected ecosystems.

So what does this all mean? Why is it important? What it tells us is that testing under lab conditions is a simplification that cannot always provide a good prediction of natural conditions. And that is important, because it exposes a fundamental problem with current toxicity testing protocols. We use these protocols with the aim of protecting our environment. But in doing so ecosystem impacts are generally estimated as if the ecosystem were merely the sum total of a handful of test species exposed to a single compound in pure form under invariant lab conditions. Time and time again, though, we have demonstrated this just does not add up – what we have here is an enormous gap in our knowledge. Thankfully, however, there is a growing realisation we need to test more than just individual species and factor abiotic fluctuations into the equation.

3. The dance of the nanomaterials

The second choreography concerns synthetic nanomaterials, microscopic particles that are today used in a growing number of applications and are claimed to make products more sustainable. Nanotechnology has been identified as a key enabling technology that brings prosperity and innovation within a wide range of commercial and industrial applications. Manipulating matter at the nano-scale (10^{-9} m) is now technically possible for virtually every material. The nano-scale gives unique properties compared to larger-sized particles of the same composition, as particles are more reactive, thus enhancing, for instance, magnetic, electrical and optical properties. The demand for engineered nanomaterials has constantly grown by 22% per year between 2014 and 2016. Virtually any material can now be made at the nano-scale and such materials are today part and parcel of everyday technologies. But as we know from the past industrial era, in which myriad new chemicals were synthesised, anything new can have hidden drawbacks and dangers. Although growing concerns have led to many studies on nanotoxicology, the mechanisms underlying the toxicity of this new class of materials are still unclear. So once again we face the crucial question: do synthetic nanomaterials pose an insurmountable long-term risk to the environment?

Consider the example of sports socks, a product in which silver nanoparticles are now being incorporated to banish sweaty odours. Every time these socks are washed, nanoparticles of silver are flushed down the drain with the rinse water. This is a good example of dissipative dispersal: something that can scarcely be avoided without simply prohibiting the use of nano-silver in socks. The rinse water ends up in a water treatment plant and subsequently in surface waters, where it's subject to various processes studied by many different research groups. It is known (e.g. citations mentioned within Savolinen et al., 2013; Xiao et al., 2018) shows that exposure conditions are of major influence on the behaviour and fate of nanomaterials. This dependence on hydrochemistry means the nanoparticles are subject to constant change, resulting in enormous fluctuations in exposure for all the elements of the ecosystem, where multiple species of plants and animals co-exist, both in the water column and in the bottom sediment where the heavier, clumped nanoparticles end up. The next question is whether the nanoparticles also have a tendency to adsorb to organisms – stick to their tissues, in other words. Many studies are now performed, as an example in five-day-old larvae we saw the intestinal walls were covered with nanoparticles (Van Pomeroy et al., 2017). By experimenting with particles of different sizes we were able to conclude that particles larger than 50 nm stuck to the

intestinal walls, while smaller particles passed through the intestinal tract. These then shot into the blood flow and we found them throughout the body, in fatty organs and tissues and even in the eyes (Van Pomeroy et al., 2017). The neonates within the brood pouch of pregnant water fleas were shown to be covered by fluorescently dyed nanoparticles (Brun et al., 2017). From another study (Cui et al., 2017) it could be concluded that when a pregnant female is exposed to nanomaterials, the offspring get off to a difficult start in life. But does it in fact matter if these materials are taken up by organisms? Current ecotoxicological lab tests employ fairly high doses, and to date we have found virtually no *acute* effects in organisms that are exposed *briefly* to nanoparticles. As yet, the impact of long-term exposure to low doses remains currently still under-explored. One major puzzle we want to clear up in work we are doing as part of an international consortium is to further translate the subtle responses exhibited by organisms to apical toxicity endpoints at the species, population and community level using mechanistic methods including Adverse Outcome Pathways (Ankley et al., 2010). The impact of nanomaterials on biotic interactions is an issue we have only just started unravelling (EU Horizon PATROLS consortium <https://www.patrols-h2020.eu/>). The first experimental step “en route” to working under semi-field conditions has thus been taken, seeking to work out the long-term impact of nanomaterials under natural conditions.

4. Predicting future impacts

Precisely with emerging technologies using new synthetic chemicals and other materials, where emissions are still negligible, it is vitally important to get to grips with potential adverse impacts before widespread environmental dispersion of the materials occurs. In the case of nanotechnology it is often entirely new substances and products that are being developed, with sustainability claims often prominently touted in the development phase. But are these new technologies indeed more sustainable in actual practice (Pallas et al., 2018)?

An illustration is the recent technological development using tandem solar cells with embedded nanomaterials that have a claimed 20–50% higher efficiencies and conversion rates of solar radiation to electricity as compared to current commercial technologies (Borgström et al., 2018). The building elements (basic principles) for the Nano-Tandem cell have been demonstrated in the laboratory and the objectives for the new tandem solar cell are clearly formulated in accordance to the definition of Technological Readiness Level 2 (European Commission, 2014). Within the project an ex ante LCA as well as an ex ante RA for the new materials are prepared as a safe-by-design challenge and to identify early stage adaptation of the cell design in order to circumvent in an early stage of development. The LCA research question was to quantify if the newly developed nanotandem PV-cells technology has lower environmental impacts compared to common monolayer silicon PV technologies. The initial pilot-scale LCA at the laboratory scale showed that carbon emissions induce the majority of the environmental impacts (Pallas et al., 2019). These high carbon emissions were for a large part attributable to the cradle to gate energy costs of the nanowires. Characterization models and factors are missing for all nanomaterials, thus it was not possible to evaluate the inherent toxicity of the materials across the life cycle (Pallas et al., 2018). This is a serious limitation with consequences for the validity of the results and conclusions of impacts. Upscaling to industrialised scales is not yet done, and comes with large uncertainties.

The RA research question was to generate the dose-response curve for the GaInP and GaAsP nanowires as used in the newly

developed Nano-Tandem cell technology. Literature on GaInP and GaAsP nanowires is scarce and hence emission, fate and toxicity assessments cannot yet be performed with a sufficient level of reliability. The growth of ternary nanowire materials like GaInP and GaAsP is costly, time-consuming and only feasible at pilot stage controlled conditions. Thus insufficient amounts of material are available to test these wires in toxicity assays. Therefore prospective assessments on risk need to be done based on dose-response curves obtained from nanoparticles that have similarities to those nanowires that need to be embedded within the Nano-Tandem cell.

This clearly shows that future scenarios must be predicted on the basis of scant knowledge and data, which means there are major inherent uncertainties. Here we are up against the Collingridge Dilemma (Collingridge, 1980). These uncertainties about the future are reduced once the technology reaches maturity – but that is in hindsight. As humans, only rarely do we foresee the consequences of our actions. What we do want to do, though, is anticipate the potential risks posed by a new technology, whether it be nanoparticles or other emerging materials. This is the kind of “into-the-future” analysis we are doing, by applying a combination of risk assessment and life cycle analysis to the entire supply chain (Guinée et al., 2017).

5. The event continuum

This takes me back to the early 1960s, when Rachel Carson was already sounding the alarm, showing that contemporary use of pesticides – or as she preferred to call them: biocides – were having a far greater impact on the natural world than was initially thought. With the knowledge she shared with the world in her book *Silent Spring* (1962) she was ultimately responsible for getting certain stipulations included in major U.S. policy documents on air quality (the Clean Air Act 1963), nature conservation (the Wilderness Act 1964; the Endangered Species Act 1972) and the broader environment (the Environmental Policy Act 1969). Her work resonated beyond America, prompting all kinds of work by the Centre for Ecology and Hydrology in the UK as well as RIVM in the Netherlands, with the upshot that today the environment is protected as well as can be protected reasonably well by a plethora of policies and legislation. Rachel Carson was not only instrumental in creating awareness of the dark side of progress but also presented us with two perspectives: the chemical road and “the other road”. And note that when doing research as described within the two storylines of the dances, we refine this way our “Wizard” knowledge on safe-by-design of “the chemical road” not necessarily addressing knowledge needed for “the other road”. The other road, which means working together with nature, manipulating a little here and there, but not appropriating nature in its entirety, which is the road to destruction. This implies discontinuing use of many of the substances we have created, which means opting for the “Prophets” sustainable, ecological road so much discussed today and already advocated by Carson back in 1962. Is this discussion that started in the 60s now again discussed being an event continuum?

6. Refrain

We can only conclude there is a lot of “matter out of place” and that it is by no means straightforward to assess *where* all the chemicals and other synthetic materials end up, *how many* of them there are, and what *risk* they pose to the ecosystem. Prior to the 1960s we were merely observers of the dance, with nature the choreographer. We stood enthralled by the mysteries around us and were shocked when our actions had an impact. Today, we see

ourselves as the choreographer, with the world deemed under our control. We synthesise myriad chemicals, convinced we are in charge of the tempo and direction of the dance. But the dance we are putting on is geared solely to the performance itself, towards securing a single specific target. To my mind we are forgetting three things. First, that we are not the only ones involved, but that we need to collaborate with a choreographer called “nature” that is orders of magnitude greater than ourselves. Second, that a good choreographer should make conscious choices about the story she wants to tell – which means giving explicitly thought to the number of actors (chemicals) on the stage, what dance steps they are to make (the environmental behaviour), at what point the climax takes place (the target) and what segments of the audience need catering to most (the environmental compartments). And third: what we are going to do when the curtain falls – when the target has been reached by the chemical – but we discover the performance has not yet ended, potentially with major consequences.

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