The risks of the herbicide 2,4-D

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Lars Neumeister

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Author: Lars Neumeister, independent pesticide expert

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Testbiotech
Institute for Independent Impact Assessment in Biotechnology
Frohschammerstr. 14
D-80807 Munich
Tel.: +49 (0) 89 358 992 76
Fax: +49 (0) 89 359 66 22
info@testbiotech.org
www.testbiotech.org
Executive Director: Dr. Christoph Then

Content

Publishers´ foreword

This is a detailed report on the herbicide 2,4-D. The background of this report is a steady increase of applications for genetically engineered herbicide resistant (also known as herbicide tolerant) plants for import into the EU that mirrors an increased interest in the cultivation of plants with resistance against the herbicide 2,4-D and others in regions as such the US, Brazil and Argentina. More than ten years into the large-scale cultivation of genetically engineered plants that are mostly resistant to glyphosate, we are seeing a strong increase in herbicide resistant plants[1] as well as in usage of glyphosate[2]. There are in addition strong indications of an increase in residues from spraying in the plants[3].

Looking at applications for genetically engineered plants currently pending in the EU[4] and other parts of the world, it shows that plants are being engineered to be resistant to more and more herbicides. Many of the plants are engineered to be resistant to glyphosate, but we are also seeing applications for plants that are being made resistant to eight other herbicides or groups of herbicides such as glufosinate, AOPPs (also known as FOPs), dicamba, ALS inhibitors, imidazolinone, isoxaflutole, mesotrione and 2,4-D. Some of these herbicides are known to be toxic, for instance, glufosinate, quizalofop (group of AOPPs) and isoxaflutole. Some plants have been engineered to be resistant to several herbicides at once.

As a result, we will see an increase in the load of residues in the food chain. The usage of dicamba in genetically engineered plants, for instance, requires higher maximum residue levels in the plants[5] and will also increase the load of carcinogenic substances like formaldehyde[6] which is one of the metabolites of dicamba.

2,4-D is known from its use as an compound (together with 2,4,5-T) Agent Orange in the Vietnam War. At that time, the most visible detrimental effects on human health were caused by dioxin, which is a highly toxic byproduct. Dioxin was classified a human carcinogen in 1997 by IARC after a long campaign by industry to stop the classification[7]. It is also capable of causing reproductive problems and damaging the immune system. As this report shows, high levels of dioxin can still be found in some 2,4-D mixtures.

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[1] Benbrook, CM (2012): Impacts of genetically engineered crops on pesticide use in the U.S. -- the first sixteen years. Environmental Sciences Europe 24(1):1-13.
[2] See above
[3] http://www.testbiotech.de/en/node/926
[4] http://registerofquestions.efsa.europa.eu/roqFrontend/questionsListLoader?unit=GMO
[5] EFSA PPR Panel (2013): Reasoned opinion on the modification of the MRL for dicamba in genetically modified soybean. EFSA Journal 2013;11(10):3440, 38 pp.
[6] EFSA GMO Panel (2013a): Scientific Opinion on application EFSA-GMO-NL-2011-93 for the placing on the market of the herbicide-tolerant genetically modified soybean MON 87708 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Monsanto. EFSA Journal 2013;11(10):3355, 30 pp. doi:10.2903/j.efsa.2013.3355
[7] Hardell L (2008): Pesticides, soft-tissue sarcoma and non-Hodgkin lymphoma - historical aspects on the precautionary principle in cancer prevention. Acta Oncologica 47: 347-354.

Furthermore, independent research is creating concerns about the risks of the active ingredient of 2,4-D for causing adverse effects in embryo development[8], birth defects[9] and endocrine disruption[10] [11]. The EU approval of 2,4-D is currently being revised and the food authority, EFSA, is carrying out a peer-review of the summary dossier prepared by German authorities. The DG SANCO standing committee will then decide on an extended approval.

There are particular concerns for users (such as farmers), rural communities and ecology in those regions where these plants are grown and sprayed with 2,4-D:

- Currently the use of 2,4-D is restricted to certain applications. In future, much larger areas will be sprayed with this herbicide, especially if 2,4-D herbicide resistant plants are grown. It is known that 2,4-D (as well as dicamba) are highly volatile and will drift by wind to other fields.[12]
- There are many mixtures of 2,4-D that can be applied, but only some of these mixtures were investigated for risks to the environment and human health. There are strong indications that the risks of several formulations have been underestimated.
- Dermal absorption after direct contact with 2,4-D (such as sprayers) is a matter of serious concern, being underestimated so far.
- Despite relevant findings, there is insufficient investigation into the effects of 2,4-D salts and esters on the potential endocrine effect on aquatic insects and the potential negative effects on human male fertility.
- Adverse effects for users (such as farmers) and the environment caused by contamination with dioxin cannot be excluded.

In the light of these findings, we demand:

- Stop extending the use of herbicide resistant plants in agriculture. Existing applications must be thoroughly reassessed for their impact on sustainable agriculture, environment and food production.
- Reject applications for commercial large-scale cultivation of plants resistant to 2,4-D because these plants will strongly increase the use of 2,4 D and therefore increase risks for farmers, rural communities and the environment.
- Suspension of 2,4-D, specifically 2,4-DMA products, until there has been a re-assessment of dermal absorption and exposure under realistic worst case scenarios (like backpack sprayer)
- A legal requirement that all pesticides should be dioxin-free (below the limits of detections, LOD). A representative number of products from all production facilities must be checked and

[8] Greenlee AR, Ellis, TM, Berg RL (2004): Low-dose agrochemicals and lawn-care pesticides induce developmental toxicity in murine preimplantation embryos. Environmental health perspectives 112(6):703-709.

[9] Schreinemachers DM (2003): Birth malformations and other adverse perinatal outcomes in four US Wheat-producing states. Environmental Health Perspectives 111(9):1259-1264.

[10] LaChapelle AM, Ruygrok ML, Toomer M, Oost JJ, Monnie ML, Swenson JA, Compton AA Stebbins-Boaz B (2007): The hormonal herbicide, 2, 4-dichlorophenoxyacetic acid, inhibits *Xenopus oocyte* maturation by targeting translational and post-translational mechanisms. Reproductive toxicology 23(1):20-31.

[11] Stürtz N, Jahn GA, Deis RP, Rettori V, Duffard RO, Evangelista de Duffard AM (2010): Effect of 2, 4-dichlorophenoxyacetic acid on milk transfer to the litter and prolactin release in lactating rats. Toxicology 271(1):13-20.

[12] Mortensen D.A., Egan J.T., Maxwell B.D., Ryan M.R., Smith R.G. (2012) Navigating a critical juncture for sustainable weed management. BioScience 2012, 62:75–84

information made available about where the samples were taken. All results must be publicly available.

- Evaluation of all 2,4-D salts and esters regarding potential endocrine effects on aquatic insects.
- Evaluation of potential negative effects on human male fertility using suitable methods.
- In depth investigation of risks of 2,4-D for embryo development, birth defects and endocrine disruption in humans.
- Obligatory and defined crop rotation for arable cropping systems to reduce weed and pest pressure.
- A shift from agricultural subsidies for unsustainable conventional agriculture to more organic agriculture and promotion of non-chemical weed control methods.

Vorwort der Herausgeber

Dieser Report befasst sich im Detail mit den Risiken des Unkrautvernichtungsmittels 2,4-D. Anlass für den Bericht ist die zunehmende Zahl von Zulassungsanträgen für den Import gentechnisch veränderter Pflanzen in die EU, die gegen u.a. Herbizide wie 2,4 D resistent gemacht wurden. Diese Anträge spiegeln das wachsende Interesse wider, derartige Pflanzen in Ländern wie den USA, Brasilien und Argentinien anzubauen. Nach mehr als zehn Jahren des großflächigen Anbaus gentechnisch veränderter Pflanzen, die hauptsächlich gegen Glyphosat resistent sind, sehen wir hier nicht nur diese deutliche Zunahme derartiger Pflanzen im Anbau[13], sondern auch eine Zunahme der Glyphosatanwendungen.[14] Zudem gibt es deutliche Hinweise auf eine wachsende Belastung der Pflanzen mit Rückständen aus diesen Spritzmitteln.[15]

Die Analyse der Zulassungsanträge, die derzeit in der EU[16] ebenso wie in anderen Ländern der Welt anhängig sind, zeigt, dass die Gentechnik in der Landwirtschaft vor allem eingesetzt wird, um immer mehr Pflanzen gegen Spritzmittel resistent zu machen. Dabei geht es nicht nur um das Spritzmittel Glyphosat, sondern auch um acht weitere Herbizide oder Gruppen von Herbiziden wie Glufosinat, AOPPs (auch als FOPs bekannt), Dicamba, ALS inhibitors, Imidazolinon, Isoxaflutol, Mesotrione und 2,4-D. Von einigen dieser Herbizide ist bekannt, dass sie sehr giftig sind, wie zum Beispiel Glufosinat, Quizalofop (aus der Gruppe der AOPPs) und Isoxaflutol. Etliche der gentechnisch veränderten Pflanzen sind gegen mehrere Herbizide gleichzeitig resistent .

Im Ergebnis ist eine steigende Belastung der Nahrungskette mit Rückständen zu erwarten. Zum Beispiel wurden für die Anwendung von Dicamba auf gentechnisch veränderten Pflanzen die Rückstandshöchstgehalte[17] erhöht.Gleichzeitig ist zu erwarten, dass auch krebserregende Rückstände wie Formaldehyd[18] zunehmen, die beim Abbau von Dicamba entstehen.

Das Unkrautvernichtungsmittel 2,4-D war (neben 2,4,5-T) Bestandteil des Entlaubungsmittels Agent Orange, das während des Vietnam-Kriegs eingesetzt wurde. Die offensichtlich verheerende Wirkung auf die menschliche Gesundheit wurde damals vor allem durch Dioxine verursacht, die als hochgiftiges Nebenprodukt bei der Herstellung von Bei 2,4-D (und 2,4,5-T) auftreten. Dioxin wird seit 1997 als krebserregend eingestuft, obwohl die Industrie lange versucht hat, diese Klassifizierung zu verhindern.[19] Zudem sind sowohl Schäden für Embryonen als auch für das Immunsystem zu befürchten. Wie dieser Bericht zeigt, können in manchen Mischungen von 2,4-D immer noch hohe Mengen an Dioxin gefunden werden.

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[13] Benbrook, CM (2012): Impacts of genetically engineered crops on pesticide use in the U.S. -- the first sixteen years. Environmental Sciences Europe 24(1):1-13.

[14] Ebd.

[15] http://www.testbiotech.de/en/node/926

[16] http://registerofquestions.efsa.europa.eu/roqFrontend/questionsListLoader?unit=GMO

[17] EFSA PPR Panel (2013): Reasoned opinion on the modification of the MRL for dicamba in genetically modified soybean. EFSA Journal 2013;11(10):3440, 38 pp.

[18] EFSA GMO Panel (2013a), Scientific Opinion on application EFSA-GMO-NL-2011-93 for the placing on the market of the herbicide-tolerant genetically modified soybean MON 87708 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Monsanto. *EFSA Journal* 2013;11(10):3355, 30 pp. doi:10.2903/j.efsa.2013.3355.

[19] Hardell L (2008): Pesticides, soft-tissue sarcoma and non-Hodgkin lymphoma - historical aspects on the precautionary principle in cancer prevention. Acta Oncologica 47: 347-354.

Zudem zeigen unabhängige Untersuchungen, dass es auch Hinweise auf Schädigung durch 2,4-D selbst gibt. Diese betreffen die embryonale Entwicklung[20], Geburtsschäden[21] und den Hormonstoffwechsel.[22] [23]

Derzeit führt die EU eine Überprüfung der Zulassung von 2,4-D durch. Die Europäische Lebensmittelbehörde EFSA bewertet dabei ein Dossier, das von den deutschen Behörden erstellt wurde. Danach wird die EU über eine Verlängerung der Zulassung entscheiden. Es gibt erhebliche Bedenken im Hinblick auf die Risiken für die Anwender (wie Landwirte), die Landbevölkerung und die Umwelt in den Gegenden, in denen die herbizidresistenten Pflanzen angebaut und mit 2,4-D gespritzt werden:

1. Derzeit ist der Gebrauch von 2,4-D auf bestimmte Anwendungen beschränkt. In der Zukunft würden wesentlich größere Flächen mit dem Herbizid gespritzt, wenn entsprechende herbizidresistente Pflanzen angebaut werden. Es ist bekannt, dass 2,4-D (genauso wie Dicamba) auch sehr leicht mit dem Wind auf die Nachbarfelder verfrachtet werden kann.[24]
2. Es gibt viele Mischungen von 2,4-D, die angewendet werden können, aber nur einige von ihnen wurden auf Risiken für Mensch und Umwelt getestet. Es gibt deutliche Hinweise darauf, dass die Risiken verschiedener Mischungen unterschätzt werden.
3. Das Risiko für einer direkte Aufnahme von 2,4-D über die Haut (u. a. beim Sprühen) scheint größer als bisher angenommen und gibt besonderen Anlass zur Sorge.
4. Es gibt trotz entsprechender Hinweise, keine ausreichenden Untersuchungen der Auswirkungen von 2,4-D-Salzen und -Estern auf mögliche hormonelle Effekte bei Wasserorganismen und auf die männliche Fruchtbarkeit.
5. Schäden durch Verunreinigungen mit Dioxinen für die Gesundheit und die Umwelt können nicht ausgeschlossen werden.

Vor diesem Hintergrund fordern die beteiligten Organisationen:

- Die Ausweitung des Anbaus von herbizidresistenten Pflanzen in der Landwirtschaft sollte gestoppt werden. Bestehende Marktzulassungen müssen im Hinblick auf ihre Auswirkungen auf die nachhaltige Landwirtschaft, Umwelt und Lebensmittelproduktion gründlich überprüft werden.
- Die Anträge auf den kommerziellen Anbau von Pflanzen, die gegen 2,4-D resistent gemacht wurden, sollten zurückgewiesen werden, weil diese zu einem starken Anstieg der Nutzung des Spritzmittels führen würden und sich damit auch die Risiken für Landwirte, die Landbevölkerung und die Umwelt erhöhen.
- Die bestehenden Zulassungen von 2,4-D und insbesondere von 2,4-DMA-Produkten sollte ausgesetzt werden, bis eine Neubewertung der Risiken erfolgt ist. Dabei muss auch die

[20] Greenlee AR, Ellis, TM, Berg RL (2004): Low-dose agrochemicals and lawn-care pesticides induce developmental toxicity in murine preimplantation embryos. Environmental health perspectives 112(6):703-709.
[21] Schreinemachers DM (2003): Birth malformations and other adverse perinatal outcomes in four US Wheat-producing states. Environmental Health Perspectives 111(9):1259-1264.
[22] LaChapelle AM, Ruygrok ML, Toomer M, Oost JJ, Monnie ML, Swenson JA, Compton AA Stebbins-Boaz B (2007): The hormonal herbicide, 2, 4-dichlorophenoxyacetic acid, inhibits *Xenopus oocyte* maturation by targeting translational and post-translational mechanisms. Reproductive toxicology 23(1):20-31.
[23] Stürtz N, Jahn GA, Deis RP, Rettori V, Duffard RO, Evangelista de Duffard AM (2010): Effect of 2, 4-dichlorophenoxyacetic acid on milk transfer to the litter and prolactin release in lactating rats. Toxicology 271(1):13-20.
[24] Mortensen D. A., Egan J. T., Maxwell B. D., Ryan M. R., Smith R. G., Navigating a critical juncture for sustainable weed management. *BioScience* 2012, 62:75–84.

Aufnahme des Wirkstoffs durch die Haut eingehend geprüft werden, unter Berücksichtigung von realistischen Szenarien wie einer Anwendung über Rucksack-Sprüher.

- Es muss gesetzlich vorgeschrieben werden, dass alle Pestizide frei von Dioxinen sein müssen. Dazu muss auch festgelegt werden, dass eine repräsentative Zahl von Produkten aller Hersteller überprüft und die Details der Überprüfung sowie ihre Ergebnisse öffentlich gemacht werden.
- Alle Salze und Ester von 2,4-D müssen im Hinblick auf ihre hormonellen Effekte auf aquatische Organismen überprüft werden.
- Die Auswirkungen auf die männliche Fruchtbarkeit müssen mit geeigneten Methoden überprüft werden.
- Die möglichen Auswirkungen von 2,4-D auf die Entwicklung des Embryos, auf Geburtsschäden und das Hormonsystem des Menschen müssen im Detail untersucht werden.
- Ein Fruchtwechsel auf landwirtschaftlichen Nutzflächen muss vorgeschrieben werden, um der Ausbreitung von Unkräutern und Schädlingen entgegenzuwirken.
- Bei der Vergabe staatlicher Subventionen müssen die Ökologisierung der Landwirtschaft und pestizidfreie Methoden zur Bekämpfung von Unkraut stärker berücksichtigt werden.

Summary

The herbicide 2,4-D is one of the oldest synthetic pesticides. It was placed on the market in the 1940ies and became infamous as part of the defoliation chemical ‘Agent Orange’ in the Vietnam War. It is still widely used all over the world.

In 2011, the US Department of Agriculture received a proposal from one of the main 2,4-D producers, Dow AgroSciences for soybeans and corn which has been genetically engineered to tolerate 2,4-D and other herbicides. The modified plants are suggested as a solution against so called superweeds which have become resistant against the herbicide glyphosate.

The proposed use, especially in soybeans may increase the use of 2,4-D tremendously, and consequently adverse effects on human health and the environment may increase. This report identifies numerous gaps in the current (and ongoing) assessment of 2,4-D:

6. It is not clear if, and to what extent 2,4-D products contain impurities of highly toxic dioxins and furans.
7. the dermal absorption is largely underestimated and unknown for widely used esters and this leads
8. to a underestimation of the exposure of 2,4-D users.

These gaps are of serious concern. Dioxins and furans are human carcinogens and endocrine disruptors, persist in the environment and accumulate in the food chain. There is also evidence that dioxin concentration may multiply under sunlight.

Some studies have shown that human skin can absorb up to 80% of 2,4-D, but the risk assessment authorities consider a much lower absorption in their risk assessment. But even when a low dermal adsorption of up to 4% is considered, workers not properly protected may experience exposure above the safety levels. Especially workers using manual spraying equipment may be affected. Measurements of urinary excretion have shown large exposure of these workers, which cannot be explained by low dermal absorption.

This report is not exhaustive, there are thousands of studies on 2,4-D, and many are written by the manufacturers’ scientists or are sponsored by the manufacturers of 2,4-D. This leads to large confusion, because it can be assumed that financial interest leads to a bias towards publications which show no negative effects. In consequence, the organized confusion makes it impossible to judge the carcinogenic properties of 2,4-D, if, however products containing 2,4-D still contain dioxin impurities, these products must be considered at least as ‘possible carcinogens’ and also as endocrine disruptors, with potential effects on reproduction.

1. Introduction

In September 2010, DowAgroSciences' scientists submitted a scientific paper (Wright et al. 2010) on genetically modified plants resistant to 2,4-D and other herbicides. The authors describe these new plants as a solution against weeds which have become resistant against the herbicide glyphosate.

In October 2011, the US Department of Agriculture (USDA) received a petition from Dow AgroSciences for soybeans (DAS-44406-6) which had been genetically engineered (GE) to provide tolerance to 2,4-D, glyphosate and glufosinate (DowAgroScience 2011). Two months later USDA received another petition for corn (DAS-40278-9), genetically engineered to tolerate 2,4-D and herbicides which are aryloxyphenoxypropionate acetyl coenzyme A carboxylase inhibitors (ACCase inhibitors[25]), also known as "fop" herbicides" (DowAgroScience 2011b).

Dow AgroSciences is not the only pesticide company responding with GE plants to the new business opportunities hat come along with the rise of glyphosate resistant superweeds:

- BASF submitted a petition for soybeans tolerating all imidazolinone herbicides[26] (imazapic, imazapyr, imazamethabenz-methyl, imazethapyr, imazaquin, and imazamox),
- BayerCrop Science submitted a petition for soybeans tolerating glyphosate and isoxaflutole[27],
- Monsanto submitted a petition for crops resisting the herbicide dicamba, and
- Pioneer Hi-Bred already got permission to produce and market glyphosate and ALS-Inhibitor[28] tolerant soybeans and corn.

Some of these herbicides are highly toxic (e.g. glufosinate, quizalofop-p-tefuryl, isoxaflutole) and some even meet the EU exclusion criteria as set by regulation 1107/2009/EC. This short report focuses on one of the oldest herbicides, 2,4-D.

2,4-D has been investigated for over 70 years and the US National Library of Medicine of the National Institutes of Health alone lists 3055 publications on a search for '2,4-dichlorophenoxyacetic'.

Many of the publications are authored by the manufacturers' scientists or are sponsored by the manufacturers of 2,4-D. This leads to large confusion, because on the one hand these papers are scientific, peer reviewed papers, but on the other hand, it can be assumed that the financial interest leads to a bias towards studies showing no negative effects. A recent study by Diels et al. (2011) has shown that in studies with genetically engineered crops, there is a strong relation between funding and outcome. Some industry financed publications leave out important information, for example Ross et al. (2005), who do not mention results which show a high dermal uptake of 2,4-D, others like Burns & Swaen (2012) include so many other industry funded studies without indicating them, that this kind of review leads to even more confusion. Basically, the industry funded/authored studies lead to a 'dilution' of information - a tactic also applied by the tobacco industry. The parallels between the tobacco industry and the pesticide industry are manifold, not only in their argumentation and strategy, but also in the final results – despite the evidence - cancer causing agents are not prohibited.

This report tries to highlight some gaps in the assessment of 2,4-D. Due to the large number of studies it cannot be exhaustive, therefore it focuses on aspects which might not have been covered before.

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[25] More info: http://www.hracglobal.com/Publications/ClassificationofHerbicideSiteofAction.aspx
[26] http://www.aphis.usda.gov/publications/biotechnology/2012/basf_soybean.pdf
[27] http://www.aphis.usda.gov/brs/fedregister/BRS_20120713l.pdf
[28] Inhibition of acetolactate synthase (ALS) see: HRAC Group B: http://www.hracglobal.com/Publications/ClassificationofHerbicideSiteofAction.aspx

2. The herbicide 2,4-D

2,4-dichlorophenoxyacetic acid (CAS 94-75-7) was first described in 1942 as a synthetic auxin, which is a class of plant hormones. The substance is used as a systemic herbicide and acts as a growth inhibitor as seen by curling leaves. The salts are readily absorbed by the roots while the esters (see below) are mostly absorbed by foliage.

It controls broad-leaved weeds (dicotyledons) while monocotyledons such as cereals (incl. maize/corn) and grass (incl. sugarcane and bamboo) are mostly unaffected (oPM 2012). The natural tolerance of monocotyledons is somewhat limited, for example in later life stages of corn plants.

The Compendium of Pesticide Common Names[29] lists for 2,4-D 29 salts and esters, while the online Pesticide Manual of the British Crop Protection Council (BCPC) lists 15 salts and esters (oPM, 2012) (see Annex I). According to Dow AgroSciences, the salt 2,4-D-dimethylammonium (2,4-DMA) and the ester 2,4-D-2-ethylhexyl (2,4-D EHE) present 90-95% of the marketed formulations (Charles et al., 2001). For the genetically engineered crops Dow AgroScience developed a new formulation of 2,4-D using a choline salt. This new formulation of 2,4-D is chemically identified as 2,4-dichlorophenoxyacetic acid (2-hydroxyethyl) trimethylammonium salt.

The BCPC pesticide manual shows that most products contain the salt 2,4-DMA , the pure acid and the ester 2,4-D EHE (oPM, 2012) (see figure 1).

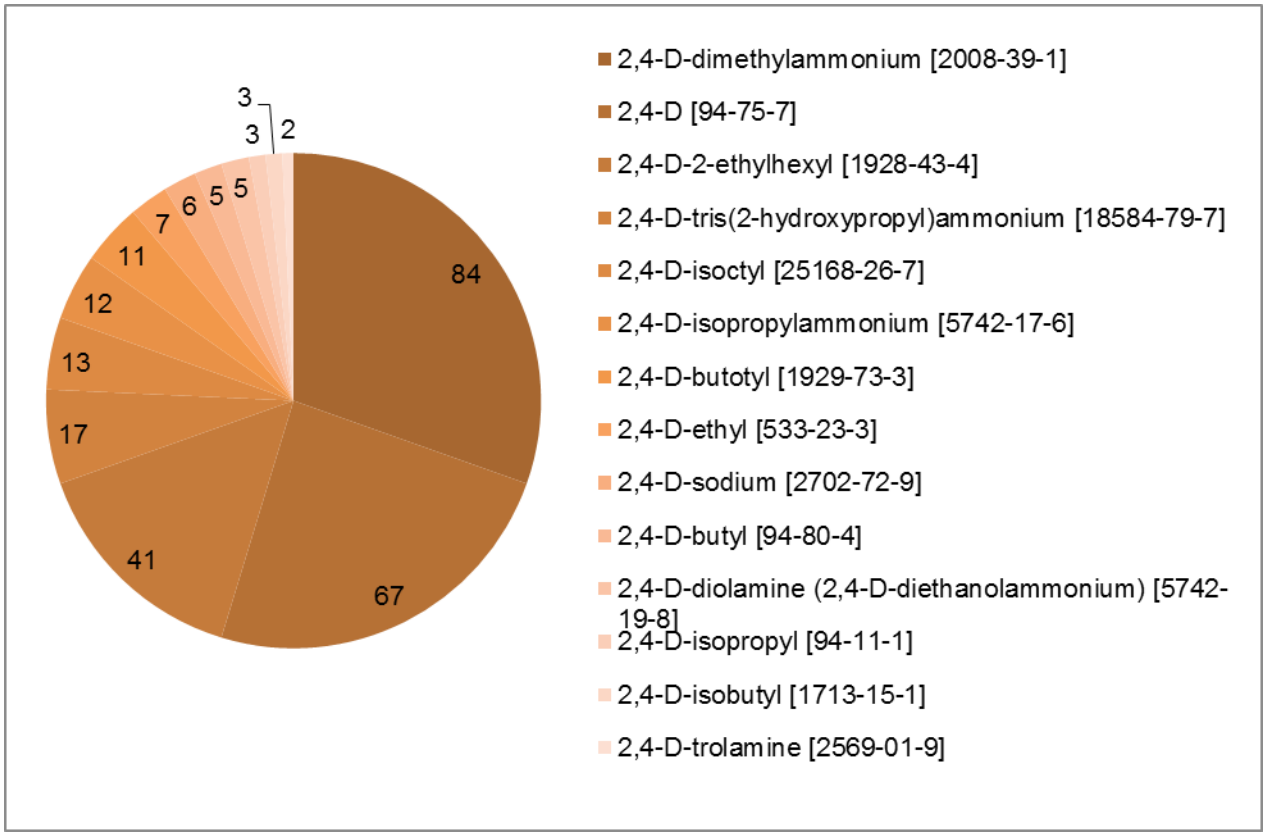

Figure 1: Number of products by formulation (own graphic based on [oPM, 2012])

[29] http://www.alanwood.net/pesticides/2,4-d.html

There are at least 75 pesticide manufacturers which market products containing 2,4-D derivates. Nufarm and Dow AgroSciences have the largest portfolio (ibid.) (see figure 2), but the number of products does not represent the market share.

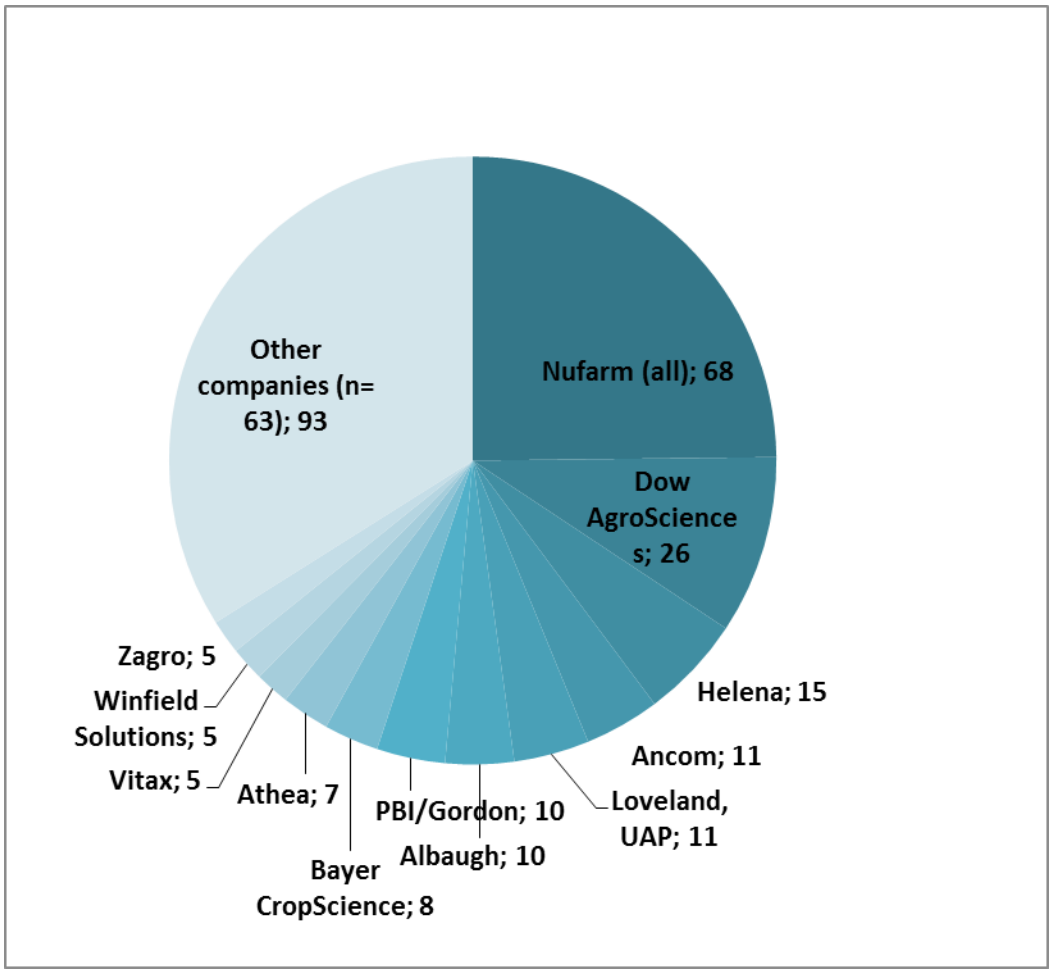

Figure 2: Number of products containing 2,4-D derivates by company

2,4-D is produced from 2,4-dichlorophenol, which is also an impurity in 2,4-D products and a relevant breakdown product/metabolite. 2,4-D is part of the group of chlorophenoxy-substances such as MCPA, MCPP, 2,4-DB and 2,4,5-T and the IARC classified them as a group of carcinogenic class 2B, "*possible carcinogenic for humans*" (IARC 2013a). Manufacturers claim that the classification is unjustified since 2,4-D is not genotoxic and claim the genotoxic impurities dioxins/furanes (phenolic impurities in 2,4-D could give rise to dioxins and furanes by condensation reactions) are not detected above the limit of quantification (LOQ). This is only partly true as described in Chapter 6.

3. The potential future use of 2,4-D in the US

Dow AgroSciences submitted a proposal for genetically engineered (GE) corn and soybeans, therefore the technology will be limited to these crops, if not extended. The main reason for the proposal is the increasing resistance of weeds to glyphosate, the major herbicide currently used in soy and corn in the USA.

So far, due to the dominance of other herbicides (glyphosate, atrazine etc.), 2,4-D was applied on less than 8 percent of the corn acreage in 2005[30] and in soy beans (2006) only on 3 percent[31] of the area.

Obviously, Dow AgroSciences developed the Enlist technology to make profit, thus the company expects an increasing use of their 2,4-D formulations. However, the scale of the potential increase is difficult to estimate.

A new report by Stratus research states that nearly half (49%) of all US farmers surveyed said they have glyphosate resistant weeds on their farm in 2012[32]. Extrapolated to area this could affect up to 61 million acres[33]. However, Dow AgroSciences is not the only company seeing a marketing opportunity in glyphosate resistant weeds, and the 2,4-D resistant plant varieties are '*stacked*' - they have multiple resistances to other herbicides (see Introduction).

Whether or not glyphosate will be completely substituted by 2,4-D (or other herbicides) will depend on the price of the GE crop & herbicide packages, and on the further development of the resistant weeds. In a complete substitution scenario about 100 million acres could be affected – that is the size of GE corn and soybean under RoundUp Ready. But is it likely that any other GE crop & herbicide package can compete with Monsantos' RoundUp Ready also on areas still free from 'superweeds'?

It is very likely, that the future use of 2,4-D (plus other herbicides) will be limited to areas where weed resistance plays a role, and in these areas Enlist competes with the other 'solutions' by BayerCropScience & Co. Nonetheless, millions of acres are already infested with superweeds, and the trend is increasing. These areas may be the target for more toxic herbicides. However, it has to be considered that some weeds already developed resistance to 2,4-D (Bernards et al., 2012), therefore 'unlimited' use does not present a long term weed control option (Egan et al. 2011). Nonetheless, the potential for Enlist (2,4-D) is large, considering the recent figures. The top soy bean producing US states are Iowa, Illinois Minnesota, Indiana, and Nebraska – states with a high incidence of superweeds and where soybean is commonly grown in rotation with corn – in these states the total 2,4-D use may therefore multiply.

The USDA draft environmental assessments (USDA 2011, 2012), do not give any number on the potential acreage for Enlist but details on the specific 2,4-D use. They anticipate a use of 2,4-D (acid equivalent) at 0.71 pound per acre, in soybean and for corn up to 3.0 pounds/acre[34]. While Dow AgroSciences assumes a 2,4-D use reduction compared to conventional corn (see figure 3, Dow AgroSciences 2011b), the USDA anticipates that application rates remain the same (USDA 2011b).

[30] http://www.aphis.usda.gov/brs/aphisdocs/09_23301p_dpra.pdf
[31] http://www.aphis.usda.gov/brs/aphisdocs/09_34901p_dea.pdf
[32] http://www.stratusresearch.com/blog07.htm
[33] 1 acre = 4047 m^2
[34] 1 pound per acre is equivalent to 1.120 kg/ha

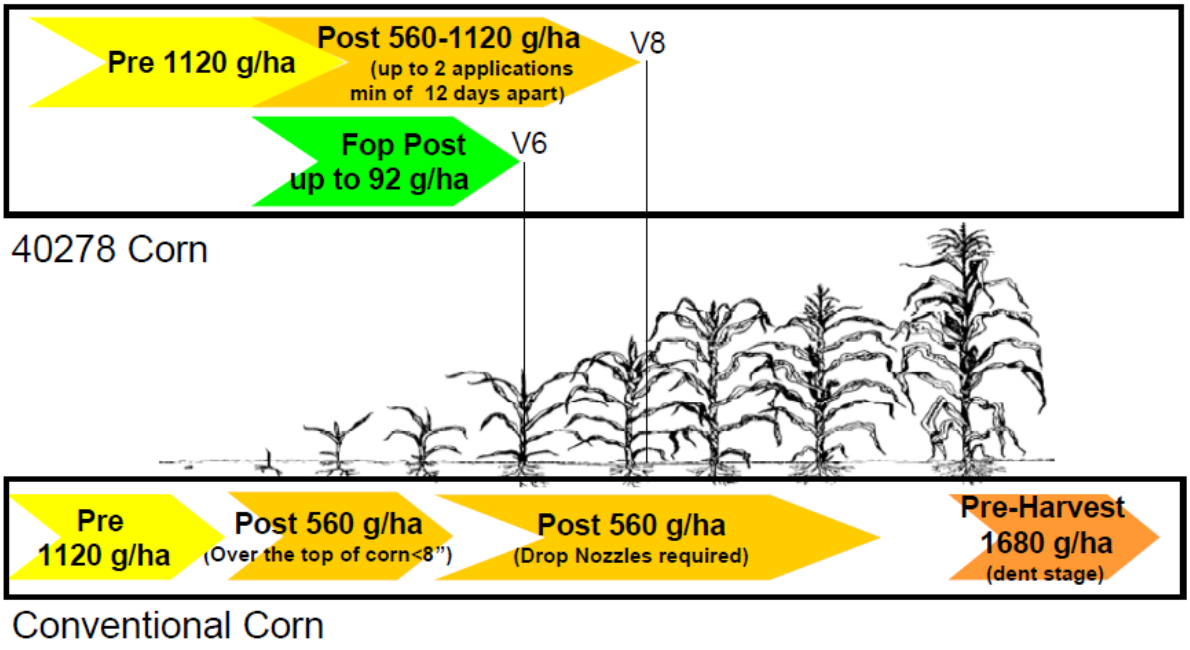

Figure 3: 2,4-D and Fop herbicide application timing and rates for conventional and DAS-40278-9 corn (Dow AgroScienes 2011)

A comparison with other GE herbicide-resistant corn, which are grown on about 70% of the corn area was not done.

4. Toxicokinetics

Humans usually absorb 2,4-D through the skin, inhale spray or to a smaller amount may swallow spray droplets. The main route of exposure is through the skin, particularly though hand exposure (Grover et al. 1986) and it seems the dermal absorption is greatly underestimated by regulators (10% by US EPA [US EPA 2012 pg. 8] and 0,08-4% by the EU [EFSA 2013a]) (see Chapter).

The intake via food should be minimal, since 2,4-D as a herbicide is mostly not sprayed over fruit and vegetables and other consumer-close products before harvest. However, residue data from 2011 from Baden-Württemberg, Northrhine-Westfalia and Saxonia-Anhaltin show that 67 samples (out of 5000[35]) contained 2,4-D residues and mostly (48 samples) on citrus fruit like oranges, grapefruit and clementine (own data, 2012). Since citrus fruit are sampled with peel, it is not clear if those residues were in the flesh/pulp and thus origin from a previous application or on the peel, which would hint to drift or postharvest exposure. The average 2,4-D concentration over all samples was low at 0,041 mg/kg.

The amounts of pesticide intake by users and bystanders depends, among others, on the adjuvants used, method of application, use intensity and the personal protective equipment (PPE) used. Regulatory risk assessment assumes that proper PPE is used and that spray equipment is in order and calibrated. However, this assumption seems to be wrong, as a large number of studies across the world show the opposite (see Matthews (2004) and Annex I in Neumeister & Isenring [2010]). Pesticide

[35] Please note that not all samples were analysed for 2,4-D.

applicators are often uneducated, do not wear PPE and use broken equipment etc. A recent survey of Malaysian rice paddy farmers using 2,4-D showed that only 14% of the 144 farmers wore gloves, and 86% wore neither shoes nor boots. (Baharuddin et al., 2011).

Based on Kohli et al. (1974) und Sauerhoff et al. (1977) it is assumed that large parts of 2,4-D are rapidly un-metabolized or bound to conjugates excreted with urine. However, pharmacokinetic studies in laboratory animals (or humans) usually use single oral or even intravenous exposure scenarios, which do not reflect occupational reality. A farmer or a hired professional applicator uses pesticides often several hours per day and in some cases 5 or more days per week. Therefore secretion of 2,4-D from real life exposure is not as fast as described in designed studies. The urinary excretion seems to more than double, when 2,4-D is applied 4-7 times versus 1-2 times (Grover et al., 1986b).

Knopp & Glass (1991) tested urine samples of forest workers applying 2,4-D with a backpack sprayer, and detected considerably amounts even 6 days (144h) post-application (see figure 4). Grover et al. (1986b) show that urinary levels after single exposure decreased to background levels on day 8 after application.

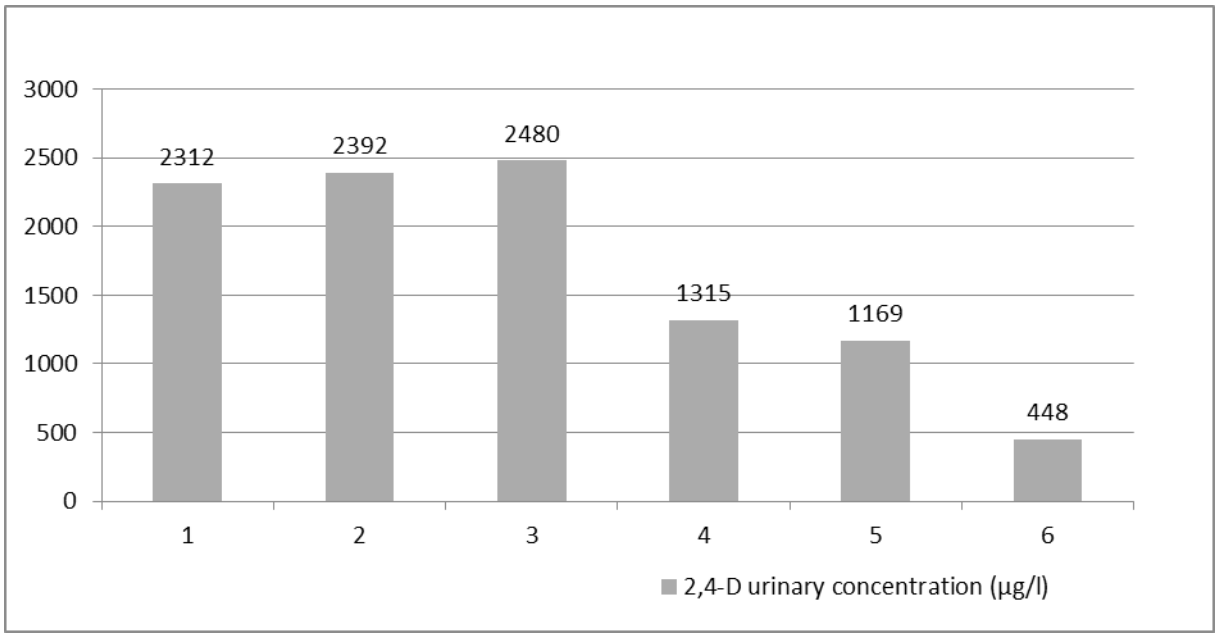

Figure 4: Urinary concentration of a sprayman 1-6 days after an 8hour occupational exposure (Data from Table 1 in Knopp & Glass 1991)

Furthermore Kohli et al. (1974) and Sauerhoff et al. (1977) applied relatively small doses, and higher doses such as during intentional or unintentional poisonings may lead to different pathways. Oliveira & Palermo-Neto (1993), for example detected 2,4-D in rats of brains fed with 10mg/kg (single dose), and suggest that 2,4-D is transported via an organic acid transportation system.

Arbuckle et al. (1999) detected 2,4-D in semen of 50% of 97 farmers who used 2,4-D before.

There is a large number of publications on the toxicokinetics and the environmental fate of 2,4-D. Many of these publications come from pesticide producers, their former employees or are sponsored by the pesticide industry.

Table 1: Publications on the toxicokinetics of 2,4-D

Dow: Sauerhoff, M.W., Braun, W.H., Blau, G.E. & Gehring, P.J. (1977): The fate of 2,4-dichlorophenoxyacetic acid (2,4-D) following oral administration to man. Toxicology 8:3–11	Human, Single 5mg/kg oral
BASF: van Ravenzwaay, B., Hardwick, T.D., Needham, D., Pethen, .S., Lappin, ? (2003): Comparative metabolism of 2,4-dichlorophenoxyacetic acid (2,4-D) in rat and dog. Xenobiotica 33(8):805-21.	Rats, Dogs Single doses 5 or 50 mg/kg
Dow: Gorzinski, S.J., Kociba, R.J., Campbell, R.A., Smith, F.A., Nolan, R.J. & Eisenbrandt, D.L. (1987): Acute, pharmacokinetic, and subchronic toxicological studies of 2,4-dichlorophenoxyacetic acid. Fundam Appl Toxicol., (3): 423-35.	Male Fischer rats, single doses oral 10-150mg/kg (pharmacokinetic study)
Former Dow employee: Timchalk, C. (2004): Comparative inter-species pharmacokinetics of phenoxyacetic acid herbicides and related organic acids. Evidence that the dog is not a relevant species for evaluation of human health risk. Toxicology, 200: 1–19.	No experiment, review
Kohli, J.D., Khanna, R.N., Gupta, B.N., Dhar, M.M., Tandon, J.S. & Sircar, K.P. (1974): Absorption and Excretion of 2,4-Dichlorophenoxyacetic Acid in Man. 4 (2): 97-100 (doi:10.3109/00498257409049349)	Human, 5mg/kg oral

A publication co-authored and partly sponsored by Dow Chemical and other 2,4-D producers claims, based on Sauerhoff et al. (1977) and Kohli et al. (1974) that, "*continuing exposure for more than 1 week of exposure would result in a steady state in which the amount excreted daily in urine would be approximately equivalent to the amount absorbed each day*." (Aylward et al., 2010, pg. 178). That seems to be a premature statement since human exposure data over a timespan longer than a week do not exist, and Knopp (1994 pg. 154.) reports from 2,4-D factory after a longer exposure pause: "*The 2,4-D urinary concentration profile for a weekly interval showed an increase in exposure during the week, culminating on Friday*." (Knopp, 1994, pg. 154). While there seems to be a certain 'equilibrium', when similar exposure continues (see second week in Figure 5), a stable 'peak' concentration could not be reached, because weekends always lowered the body burden.

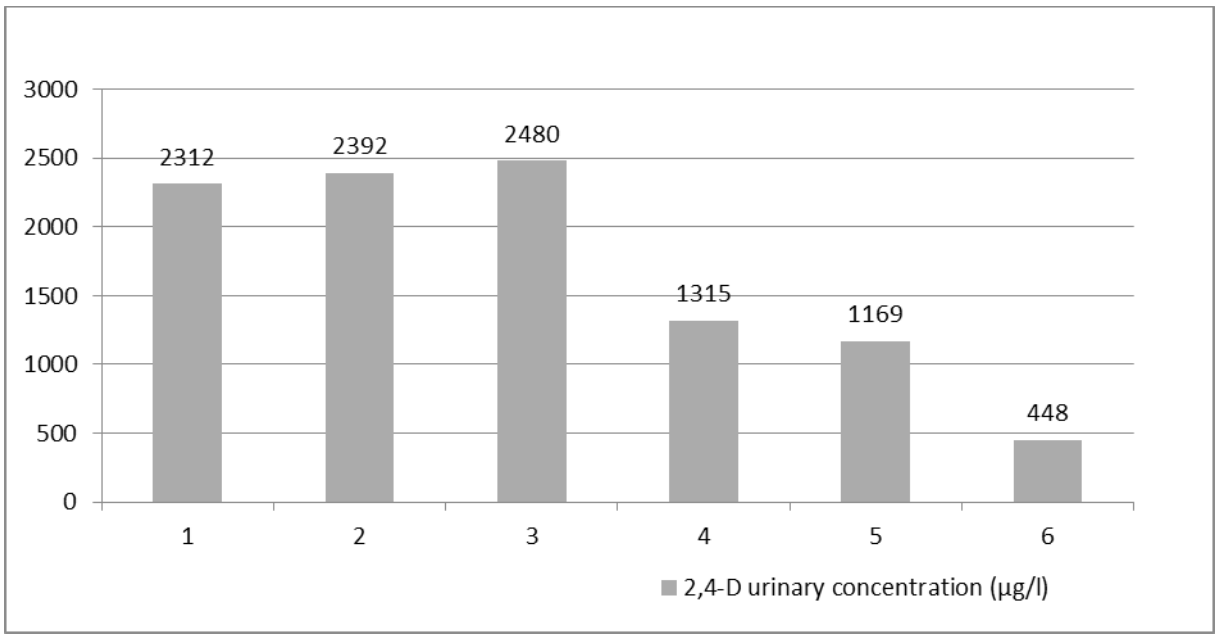

Figure 5: Mean daily urinary concentration (log µg/l) of 2,4-D over two weeks (2,4-D production workers) Data from Knopp 1994

The dermal absorption of 2,4-D and is salts and esters is underestimated

Despite the 70 year marketing history, surprisingly little is known about the dermal absorption of 2,4-D and its salts and esters. While there are some publications on trials with human volunteers, the results are inconclusive.

An industry sponsored review by Ross et al. (2005) about the dermal absorption of 2,4-D is greatly exaggerating, misleading and in part false. It states: "*The human percutaneous absorption of 2,4-dichlorophenoxyacetic acid (2,4-D) is well characterized. Five studies using human subjects have been published and the results of those studies showed remarkable reproducibility across a span of three decades and multiple laboratories, formulations, and methods*."

There are several errors in that statement:

1. The percutaneous absorption of humans exposed to 2,4-D is not well characterized. None of the two experiments with 2,4-acid was conducted with water as a vehicle, instead acetone and ethanol were used. The US EPA guideline clearly requires '*Dilutions are made with the field vehicle, usually water, to produce a solution or suspension. (…) organic solvents or special solubilizing/suspending agents must not be used*' (US EPA 1998) in tests for skin penetration. In only three experiments (Moody et al. 1990, Moody et al. 1992 and Harris & Solomon 1992) water was used as vehicle, but only one salt (2,4-DMA) was tested, and the percentage 2,4-D DMA absorbed varies between 1,76±0,6% SD and 58±23% SD (see Table 1 and Figure 6). Most of these experiments underestimate the dermal absorption because measured urinary excretion was equaled by absorption, and in some cases the time period after exposure was too short to make the excretion 100%.

None of widely marketed esters (see Figure 1), such as 2,4-D EHE has been tested. In its review report about 2,4-D the European Commission therefore points out that '*Some endpoints however may require the generation or submission of additional studies (...). This may particularly be the case for*

- *In vivo dermal absorption study in the rat with 2,4-D ester.*
- *In vitro dermal absorption study on rat and human skin with 2,4-D ester*' (EC 2001),

In their submission for the renewal of the 2,4-D authorization on EU level, the manufacturers failed to deliver this information, only results dermal absorption studies with 2,4-D acid (*in vitro*) and 2,4-D DMA (*in vivo*) were submitted (EFSA 2013b).

2. There are ***six*** studies using humans. The reviewers omit the human data of Moody et al. (1990) with the high absorption figures of up to 58±23% SD,

3. The studies Ross et al. (2005) mention cover ***two*** formulations (2,4-D acid and 2,4-DMA) while at least 12 more salts and esters are marketed (see figure 6).

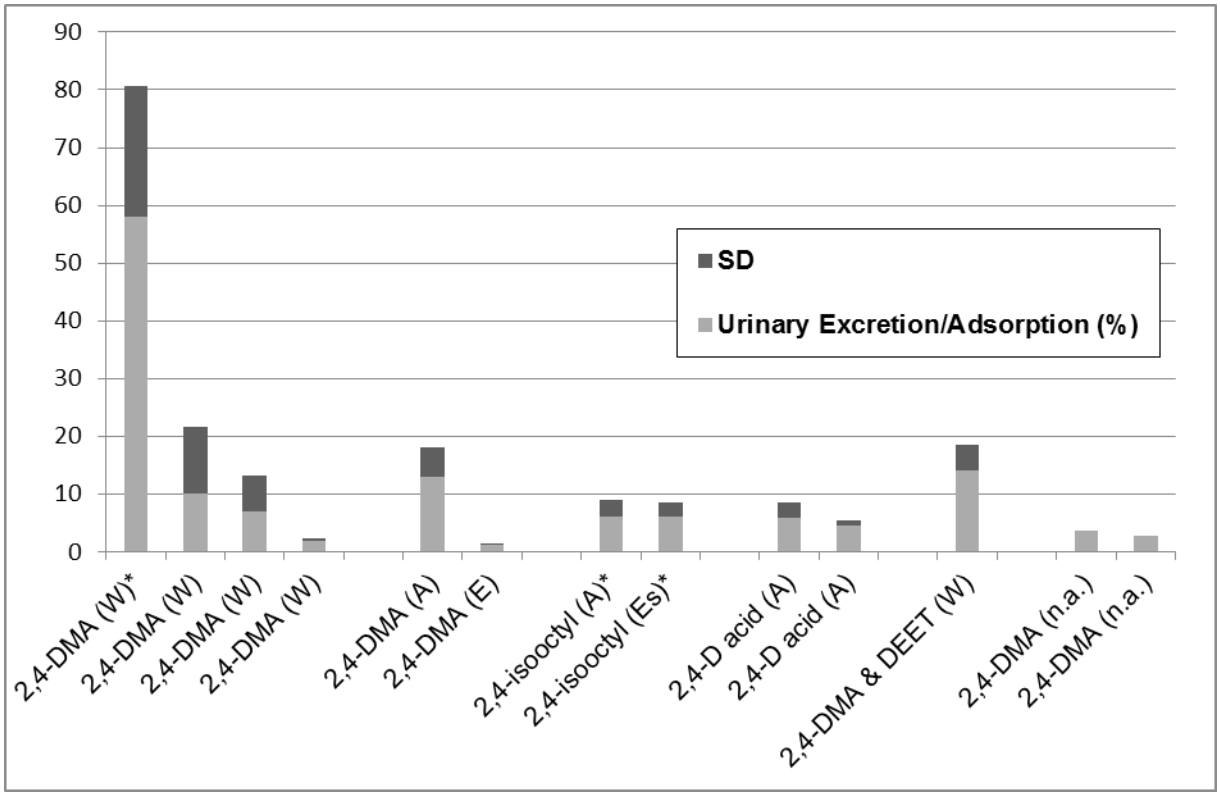

Figure 6: Dermal absorption of 2,4-D acid, 2,4-DMA and 2,4-D isooctyl by human volunteers. Vehicle in parenthesis (W= water, A= acetone, E=ethanol, Es= Esteron LV 96, n.a.= information not available). * indicates results omitted by Ross et al. 2005.

Table 2: Experiments with human dermal exposure and their uncertainties

Treatment	n	Vehicle	Site	Dose	Unit	Urinary Excretion/Adsorption (%)	SD	Duration (h)	Source	Uncertainties
2,4-DMA (W)*	6	water	Forehead (human)	4	µg/cm2	58	23	168	Moody et al. 1990	
2,4-DMA (W)	4	water	Palm (human)	1,7	µg/cm2	10	12	120	Moody et al. 1992	Urinary excretion in 120h is not equal dermal adsorption
2,4-DMA (W)	4	water	Forearm (human)	1,7	µg/cm2	7	6,2	120	Moody et al. 1992	Urinary excretion in 120h is not equal dermal adsorption
2,4-DMA (W)	5	water	Backhand (human)	1111	µg/cm2	1,76	0,6	144	Harris & Solomon 1992	
2,4-DMA (A)	4	acetone	Forearm (human)	1,7	µg/cm2	13	5	120	Moody et al. 1992	Urinary excretion in 120h is not equal dermal adsorption
2,4-DMA (E)	6	ethanol	Forearm (human)	39,67	µg/cm2	1,1	0,3	144	Wester et al. 1998	Urinary excretion extrapolated to adsorption using a Rhesus monkey as reference
2,4-isooctyl (A)*	4	acetone	Forehead (human)	4	µg/cm2	6	3	168	Moody et al. 1990	
2,4-isooctyl (Es)*	4	Esteron LV 96	Forehead (human)	4	µg/cm2	6	2,6	168	Moody et al. 1990	
2,4-D acid (A)	6	acetone	Forehead (human)	4	µg/cm2	5,8	2,8	120	Feldman & Maibach 1974	No complete excretion after 120h
2,4-D acid (A)	5	acetone	Backhand (human)	1111	µg/cm2	4,46	0,8	144	Harris & Solomon 1992	
2,4-DMA & DEET (W)	4	water	Palm (human)	1,7	µg/cm2	14	4,5	120	Moody et al. 1992	Urinary excretion in 120h is not equal dermal adsorption
2,4-DMA (n.a.)	6	n.a.	Forearm (human)	4	µg/cm2	3,6		120	Maibach &Feldman 1974 as cited Ross et al. 2005	No complete excretion after 120h
2,4-DMA (n.a.)	6	n.a.	Forearm (human)	4	µg/cm2	2,8		120	Maibach &Feldman 1974 as cited Ross et al. 2006	No complete excretion after 120h

In general, the design of dermal absorption studies is questionable. Professional users of pesticides apply them several hours per day over several days per week. Their skin is repeatedly exposed.

5. Exposure above the toxicological thresholds

In 2010, a publication co-authored and partly sponsored by 2,4-D producer concluded: "*Biomonitoring data (…) indicate that current exposures to 2,4-D are below applicable exposure guidance values*." (Aylward et al., 2010, pg. 1)

The authors collected data on urinary excretion of 2,4-D from different groups of exposed/non-exposed persons and tried to make a relation to exposure and indirectly to toxicological thresholds by comparing concentrations with so called biomonitoring equivalent (BE), an invention of Summit

Toxicology, a full service toxicology, risk assessment and pharmaceutical consulting firm[36]. Biomonitoring Equivalents (BE) are defined as the biomonitoring levels of specific chemicals in blood, urine or other human biological media or tissues that are consistent with existing exposure guidance values.

2,4-D intake is almost completely excreted via urine depending on the exposure route (oral, dermal, inhalative), the amount of intake, and the formulation. Therefore the use of urinary concentrations to measure exposure is not entirely absurd. However, in order to achieve a sound assessment, data on the ***total*** urinary excretion after the exposure has to be used and adjusted for non-urinal losses.

During the application of 2,4-D, the body adsorbs a certain percentage of the applied amount, that adsorption is finished after the exposure, and the body has a 100% 2,4-D load, which is then sequentially released from the body. Excretion of 2,4-D occurs at least up to six days after application (see figure 4) and seems to peak on the second-fourth day post application (see also Grover 1986b Fig. 1 pg. 76). It is therefore nonsense to take urinary concentration samples one day after exposure to evaluate exposure, but that is exactly what Aylward et al. (2010) do. Knopp & Glass (1982) indicate that urinary samples 24 h post application could be extrapolated to calculate total 2,4-D uptake, but this may lead to wrong numbers. In figure 7 is shown that driver A and sprayer A had similar urinary concentration 24h after application, but the total sprayer's body load is more than double as high.

Aylward et al. (2010) use data from four studies containing data on urinary concentration to assess potential occupational risks (see Figure 2 in Aylward et al [2010]). None of the studies measured the full 2,4-D excretion:

1. Curwin et al. (2005) p. 501 describe their sampling: '*The first visit was shortly after a pesticide application event (within 1–5 days) (…). Two spot urine samples on each visit were collected from the participants, one in the evening of the day of the visit, and one the following morning.'(*Curwin et al. 2005 pg. 501). This means that the actual exposure happened in a range from 1-5 days. As seen in (above) the urinary excretion can be reduced by 50% between the 1st day and the 5th day after the application.
2. In the investigation sponsored by the 2,4-D producers (Alexander et al. 2007) urine samples were collected for only 72 hours after exposure, and Aylward et al. (2010) use only urinary concentration data from the first day post-application, although excretion obviously continued and was even higher on the second day (Alexander et al. 2007).
3. Thomas et al. (2009) collected urine samples the day before application and up to four days after application. Aylward et al. (2010) used only urinary concentration data from the first day post-application, although excretion continued.
4. Arbuckle et al. (2002) sample only once, 24h after application and therefore could not measure full excretion.

In order to assess, if a toxicological threshold is exceeded, the following data should be preferably exist: data on exposure (time and duration of application, amounts used, active ingredient/formulation applied), on the pharmacokinetics (body adsorption, duration in the body, metabolization/ excretion, the valid toxicological thresholds and the bodyweight of the exposed person.

Despite the long history of 2,4-D use, not many publications provide that kind of information. Draper & Street (1982) investigated exposure of four forest workers (two drivers, two sprayers) applying 2,4-D DMA from a truck using boom jet nozzles. Sprayteam A conducted only one application, while sprayteam B continued using 2,4-D DMA daily throughout the experiment. The urinary concentrations were only measured for 72 hours post application, but as seen in the total concentration in the body was at least 4.300 µg/l for driver A and 10.000 µg/l for sprayer A.

[36] http://www.summittoxicology.com/

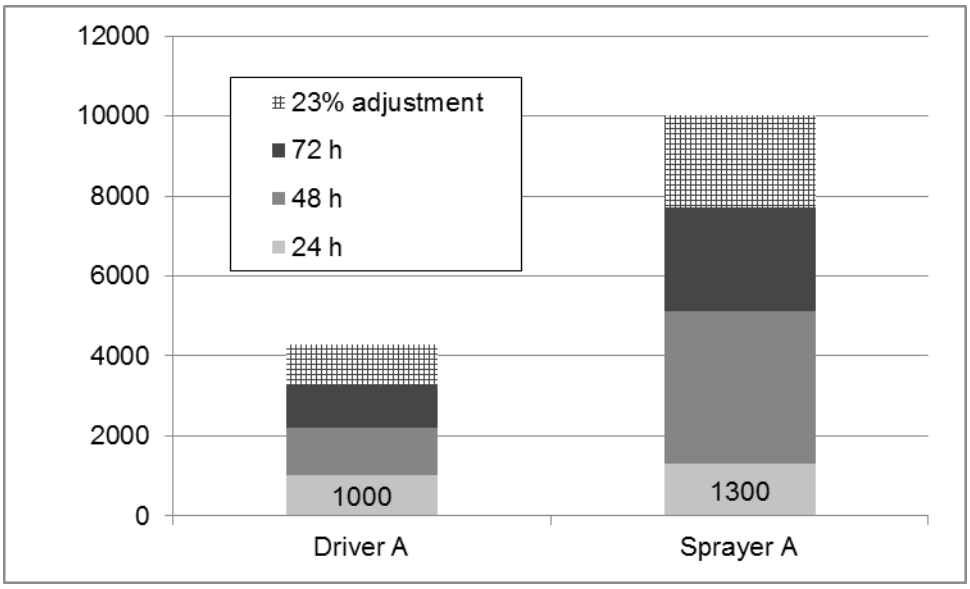

Figure 7: Urinary excretion (µg/l) of 2,4-D three days after a single spray operation of 2,4-D DMA in forest plantations (own graphic based on Draper & Street 1982 plus 23% adjustment for non-urinary loss according to Harris & Salomon 1992)

These exceed the biomonitoring equivalent (BE) by Aylward et al. (2010) by far. Draper & Street (1982) also calculated total exposure per bodyweight. For sprayer A the exposure was 160µg/kg bodyweight, which is above the Acceptable Operator Exposure Level (AOEL) of 150µg/mg bodyweight. However, the calculation of Draper & Street (1982) is an underestimation because not the full excretion was measured (ibid.).

Garry et al. (2001) and Figgs et al. (2000) report urinary concentration of up to 1700 µg 2,4-D/L for single day measurements and Zhang et al. (2011) large concentrations per spray day. None of the three research teams collected data post-application, but the spot measurements imply very high exposure. Draper & Street (1982), Garry et al. (2001) and Figgs et al. (2000) and Zhang et al. (2011) investigated only a very small number of 2,4-D applicators and among these were always some workers with extremely high urinary concentration, and apparently high exposure. These ‘outliers’ should not be neglected, because, if extrapolated, thousands of 2,4-D users could be affected.

The EFSA renewal assessment report (EFSA 2013a) comes to similar conclusions. Even when a low dermal adsorption of up to 4% is considered, potential exposure of workers not using PPE is above the Acceptable Operator Exposure Level (AOEL), although tractor mounted spraying equipment is used (details see EFSA 2013b). If basic PPE is worn the potential exposure reaches 27% of the AOEL. However, that is a large underestimation, because the dermal uptake seems to be much higher. Manual uses with backpack sprayers and/or manual boom sprayers are not considered. The EFSA should consider such high exposure groups in its risk assessment. Furthermore, use of PPE is an idealistic scenario.

6. Dioxin and 2,4-D

Production and use of 2,4-D and other chlorinated pesticides has been associated with considerable environmental contaminations with dioxins and furans. The use of Agent Orange in the Vietnam War made 2,4-D infamous especially because of the highly toxic byproduct TCDD: '*Millions of gallons of Agent Orange (a 50:50 mixture of (…) 2,4,5-T and (…) 2,4-D) used as a defoliant in the Vietnam War during 1962 to 1970 contained 2 to 30 ppm TCDD*' (NTP 2001). TCDD (2,3,7,8-Tetrachlorodibenzo-*p*-dioxin is *known to be a human carcinogen* based on sufficient evidence of carcinogenicity from studies in humans, both epidemiological and on the mechanism of carcinogenesis (ibid). It is also a potent endocrine disruptor with significant effects on reproduction (Heiden et al., 2006).

Public awareness especially after the Seveso accident[37] in 1976 made governments in Western countries act, and production and products were technically improved. In the countries of the Eastern block the problem continued much longer. Knopp (1994) reports about a 2,4-D plant in Bitterfeld in the area of former GDR: '*2,4-D produced in the chemical plant under investigation in the present study showed mainly polychlorinated diphenylether as a byproduct. Furthermore, tetrachlorodibenzodioxins (1,3,6,8-TCDD; 1,3,7,9-TCDD) were found at concentrations of 10 ppb as well as tetra and pentachlorodibenzofurans at relatively high concentrations of 400 ppb(...)*'.

Between 1978 and1997, the West-German authorities frequently tested pesticide products which potentially contain dioxin and/or furans. The last detection of dioxins or furans was in 1992, afterwards the limits of detections (LOD) of 0,01 mg/kg resp. 0,005 mg/kg were never exceeded.

In the last years, due to improved analytical methods, the limit of detection (LOD) could be significantly lowered. In 2011, authorization holders of 2,4-D, Dichlorprop-P, MCPA, Mecoprop-P and Prochloraz were requested to analyze the technical active ingredients for dioxins utilizing up-to-date methods. The results showed compliance with maximum thresholds of the regulation prohibiting certain chemicals (BVL 2013)[38]. These thresholds are shown in table 2.

Interestingly, while dioxins and furans above the thresholds are prohibited in Germany for most chemicals, the prohibition explicitly excludes pesticides[39]. On European Union level contaminations of dioxins and furans in pesticides are also not prohibited *per se*. Authorization holders are obliged to report relevant impurities, and thresholds are set on a case by case level. Currently, only one pesticide (Prochloraz) contains dioxins and furans as relevant impurities – the threshold is set at 0,01 mg/kg (WHO-PCDD/T TEQ[40]) (EC 2011).

The EU review concluded in 2001, based upon the data submitted by the 2,4-D producers, that the manufacturing impurities dioxins and furans, which are of toxicological concern, are kept at non-detectable levels (EC 2001).

[37] http://en.wikipedia.org/wiki/Seveso_disaster

[38] Answer of the Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (BVL) on request of the author 5.2.2013.

[39] http://www.gesetze-im-internet.de/chemverbotsv/BJNR172010993.html (Abschnitt 4)

[40] World Health Organization toxic equivalent (WHO- TEQ) more info: http://www.bfr.bund.de/en/questions_and_answers_on_dioxins_and_pcbs_in_food-69876.html#topic_131091

Table 3: German thresholds for dioxin and furan impurities in chemicals

Threshold for	Dioxin/ Furan	Thresholds
Sum of	2,3,7,8-Tetrachlordibenzo-p-dioxin 1,2,3,7,8-Pentachlordibenzo-p-dioxin 2,3,7,8-Tetrachlordibenzofuran 2,3,4,7,8-Pentachlordibenzofuran	1 ppb (1µg/kg)
Sum of	2,3,7,8-Tetrachlordibenzo-p-dioxin 1,2,3,7,8-Pentachlordibenzo-p-dioxin 2,3,7,8-Tetrachlordibenzofuran 2,3,4,7,8-Pentachlordibenzofuran 1,2,3,4,7,8-Hexachlordibenzo-p-dioxin 1,2,3,7,8,9-Hexachlordibenzo-p-dioxin 1,2,3,6,7,8-Hexachlordibenzo-p-dioxin 1,2,3,7,8-Pentachlordibenzofuran 1,2,3,4,7,8-Hexachlordibenzofuran 1,2,3,7,8,9-Hexachlordibenzofuran 1,2,3,6,7,8-Hexachlordibenzofuran 2,3,4,6,7,8-Hexachlordibenzofuran	5 ppb (5µg/kg)
Sum of	2,3,7,8-Tetrachlordibenzo-p-dioxin 1,2,3,7,8-Pentachlordibenzo-p-dioxin 2,3,7,8-Tetrachlordibenzofuran 2,3,4,7,8-Pentachlordibenzofuran 1,2,3,4,7,8-Hexachlordibenzo-p-dioxin 1,2,3,7,8,9-Hexachlordibenzo-p-dioxin 1,2,3,6,7,8-Hexachlordibenzo-p-dioxin 1,2,3,7,8-Pentachlordibenzofuran 1,2,3,4,7,8-Hexachlordibenzofuran 1,2,3,7,8,9-Hexachlordibenzofuran 1,2,3,6,7,8-Hexachlordibenzofuran 2,3,4,6,7,8-Hexachlordibenzofuran 1,2,3,4,6,7,8-Heptachlordibenzo-p-dioxin 1,2,3,4,6,7,8,9-Octachlordibenzo-p-dioxin 1,2,3,4,6,7,8-Heptachlordibenzofuran 1,2,3,4,7,8,9-Heptachlordibenzofuran	100ppb (100µg/kg)
Sum of	2,3,7,8-Tetrabromdibenzo-p-dioxin 1,2,3,7,8-Pentabromdibenzo-p-dioxin 2,3,7,8-Tetrabromdibenzofuran 2,3,4,7,8-Pentabromdibenzofuran	1 ppb (1µg/kg)
Sum of	2,3,7,8-Tetrabromdibenzo-p-dioxin 1,2,3,7,8-Pentabromdibenzo-p-dioxin 2,3,7,8-Tetrabromdibenzofuran 2,3,4,7,8-Pentabromdibenzofuran 1,2,3,4,7,8-Hexabromdibenzo-p-dioxin 1,2,3,7,8,9-Hexabromdibenzo-p-dioxin 1,2,3,6,7,8-Hexabromdibenzo-p-dioxin 1,2,3,7,8-Pentabromdibenzofuran	5 ppb (5µg/kg)

An investigation of two 2,4-D formulations with expiration dates in 1996 resp. 1998 in Japan showed that levels of dioxin and dioxin-like impurities were at low levels (e.g. 0,0021 µg TCDD (2,3,7,8-Tetrachlorodibenzo-*p*-dioxin /kg active ingredient) or the limit of detection, respectively (Masunaga et al., 2001).

An recent investigation by an Australian News Channel showed that one imported 2,4-product contained high levels of dioxin[41] and a recent study (Holt et al., 2010) analyzing two 2,4-D formulations in Australia concluded: '*In the present study, however, 2,4-D formulations (…) manufactured in 2006 and 2005, (…) contained TEQ levels (0.00098-0.17 ng TEQ g[42]-1 active ingredient) comparable to those manufactured 10-20 years ago (…). These results indicate that reduction measures to avoid PCDD/F impurities in pesticides are not applied or effective at all pesticide manufacturing plants*.'

The same authors also found out that under sunlight traces of dioxin contained in pesticide formulation may multiply (Holt et al., 2012). The result of Holt et al. (2010, 2012) and by the journalists are of great concern. The researcher tested only two formulation and found dioxin levels up to 0,17 ng TEQ g^{-1} active ingredient, which is above the limit of detection. Most other recent information stating that 2,4-D formulations are basically 'dioxin-free' comes from the manufacturers, but it is not clear whether or not formulations from all producing factories globally were tested. The 2013 renewal assessment report of the European Food Safety Authority (EFSA) states that all companies which are part of the European 2,4-D task force comply with the threshold of max. 10ppb (10µg/kg) TEQ (EFSA 2013a). That is no insurance for dioxin-free products. Dioxins are highly toxic, and persistent in the environment. If 2,4-D still contains quantifiable dioxin concentrations, the toxicology of 2,4-D has to be reconsidered.

7. Effects on the endocrine system

In 2000, the European Union classified 2,4-D as a potential[43] endocrine disruptor based on results derived from *in vitro* experiments (EC 2000). The European Union defines an endocrine disruptor as "*an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations*."[44] This is the same definition as set by the WHO in 1996.

One study conducted with 2,4-D shows effects which seem to meet the EU definition of an endocrine disruptor. Park et al. (2010) exposed the aquatic insect *Chironomus riparius* to low concentrations of 2,4-D and observed a statistically significant change in sex ratio of 40% male versus 60% females. In addition, a significantly higher percentage of mouthpart deformities were observed as significantly *C. riparius* exposed to 0.1 µg L^{-1} of 2,4-D compared to the control group (ibid.). A study by Rodriguez et al. (1994) also showed certain effects of 2,4-D exposure on the reproductive system of crabs (*Chasmagnathus granulate)*, but an observation of impacts on the organism or its population was not pursued.

Several studies on humans and animals report changes of hormonal levels after 2,4-D exposure, but some others (mostly conducted by Dow) did not find similar changes (see Table 4).

[41] http://www.abc.net.au/news/2013-07-22/four-corners-dangerous-dioxins/4833848

[42] 1 ng/g = 1 ppb = 1 µg/kg (footnote by author)

[43] Potential for endocrine disruption: *In vitro* data indicating potential for endocrine disruption in intact organisms. Also includes effects *in vivo* that may, or may not, be ED-mediated. May include structural analyses and metabolic considerations.

[44] http://ec.europa.eu/environment/endocrine/definitions/endodis_en.htm

Table 4: Studies on changes of hormonal levels after 2,4-D exposure

Source	Object	Observations
Garry et al. (2001)	Applicators of 2,4-D in forests	Serum luteinizing hormone (LH) values were correlated with urinary 2,4-D levels, but follicle-stimulating hormone and free and total testosterone were not. (…) herbicide applicators with high urinary levels of 2,4-D (…) exhibited elevated LH levels.
US EPA (2010) evaluation of data submitted by Dow Chemical Company.	Rats	There were no statistically significant, treatment-related differences in serum T3, T4, or TSH. At 600 ppm predicted pattern of thyroid hormone changes: decreased T3 and decreased T4 with lower TSH levels) were displayed. That suggest 2, 4-D exposure may adversely affect thyroid function at doses above the renal saturation clearance. The thyroid effects noted below renal saturation are not considered sufficiently robust to be adverse. Similar effects in first generation (F1) pups of mothers treated with high 2,4-D levels.
Xie et al. (2005)	Fish (rainbow trout)	Juvenile rainbow trout exposed to 2,4-D (1.64 mg/l) for 7 days had a 93-fold increase in plasma vitellogenin (an estrogen receptor responsive marker) levels, compared with untreated fish.
Coady et al. (2013) (Dow Chemical Company)	Fish (fathead minnows (*Pimephales promelas*))	Fathead minnows were exposed to 2,4-D concentrations 0,245; 3,14; 34,0; and 96,5 mg ae/L for 21 days. No significant differences between control and 2,4-D exposed fish in regard blood plasma concentrations of vitellogenin.
Coady et al. (2013) (Dow Chemical Company)	Tadpoles of *Xenopus laevis*	No significant histopathological effects of the thyroid gland. 2,4-D is considered ‘‘likely thyroid inactive’’ in the Amphibian Metamorphosis Assay with a No Observable Effect Concentration (NOEC) of 113 mg ae 2,4-D/L.
Rawlings et al. (1998)	Female sheep (ewes)	Sheep received 2,4-D (10 mg/kg[45]) 3 times per week into their rumen. After 36 d of treatment, blood samples were taken for hormone analysis. Serum T4 was significantly decreased, while other hormonal markers such as cortisol, estradiol, insulin and LH (luteinizing hormone) were not significantly changed compared to the control group.
Gorzinski et al. (1987) (Dow Chemical Company)	Rats	Male and female Fischer rats received dose levels of 0, 15, 60, 100, or 150 mg/kg/day of purified or technical-grade 2,4-D acid for 13 weeks. Higher dose levels of technical-grade and purified 2,4-D decreased total serum thyroxine levels in female rats, however, the morphology of the thyroid gland was normal.
Charles et al. (1996) (Dow Chemical Company)	Rats	Subchronic toxicity studies in rats were conducted on three forms of 2,4-D: the parent form, 2,4-D acid; 2,4-D dimethylamine salt (DMA); and 2,4-D 2-ethylhexyl ester (2-EHE). Doses in the subchronic studies (on an acid equivalent basis) were 0, 1, 15, 100, and 300 mg/kg/day. Major treatment related findings in the three studies included decreases in Thyroxine 3 and Thyroxine 4 levels.

Results from *in vitro* experiments are inconclusive. Kanyama et al. (2005) shows some activity of 2,4-D on human estrogen-related receptors (ERR), but at least 11 other in vitro assays do not show estrogenic activity (see Table 5). Kim et al. (2005) actually suggest androgenic actions (Kim et al., 2005).

[45] This dose is ***not*** mg per kg bodyweight as falsely interpreted by the US EPA.

Table 5: Studies demonstrating no estrogenic activity of 2,4, D *in vitro*

Reference	Bioassay
Blair et al. (2000)	Uteri cells of female rats without ovaries.
Petit et al. (1997)	Yeast and trout hepatocytes
Hurst & Sheahan (2003)	Yeast
Jung et al. (2004)	Yeast
Kojima et al., 2004	Gene assays using Chinese hamster ovary cells
Vonier et al. (1996)	Competition binding assays
Soto et al. (1995)	E-SCREEN assay using human breast cancer estrogen-sensitive cells
Jungbauer & Beck (2002)	Yeast
Lin & Garry (2000)	Human breast cancer estrogen-sensitive cells
Nishihara et al. (2000)	Yeast
Orton et al. (2009)	Two in vitro assays (yeast & cultured *Xenopus* oocytes)

8. 2,4-D and CMR properties

Before regulation 1107/2009/EC pesticides associated almost with any hazard, like carcinogenicity, mutagenicity or reproductive (CMR) toxicity could be authorized in the European Union as long as the calculated risk would be managed through certain measures/ restrictions. The new regulation 1107/2009/EC *hypothetically* abolished that kind of risk assessment/management and aims at an elimination of pesticides which are carcinogenic, mutagenic or toxic to reproduction. In reality, the European Commission in conjunction with some Member States (MS) prolonged existing authorizations for pesticides despite the fact that cut-off criteria (see Annex II and III) are met AND exposure scenarios show that critical thresholds such as the AOEL are exceeded even under “proper” use.

However, in order to meet the EU cut-off criteria regarding carcinogenicity, mutagenicity or reproductive toxicity usually *in vivo* evidence has to be delivered. *In vitro* cell studies can point in a certain directions and explain certain mechanism, but they are usually not sufficient to place a pesticide in a high category for CMR (except human germ cell studies for mutagenicity). Studies conducted with mixtures or products indicate effects caused by one or several of the ingredients (or in a synergistic or antagonist way), but the particular observed affect cannot be associated with the actual active ingredient. There are usually very many different formulations, many with unknown inert ingredients. Therefore results with a particular product cannot be considered representative for all existing products or the active ingredient. However, it is known that certain inert ingredients are very toxic themselves, while others enhance the efficacy (toxicity) of the active ingredient. In order to evaluate the properties of an active ingredient, results from studies with mixtures or formulations are not practical.

None of the regulatory agencies in Europe or the US or the IARC assigns carcinogenic potential to 2,4-D. The IARC still lists chlorophenoxy herbicides as agents that are possibly carcinogenic (IARC 2013a) or show limited evidence of carcinogenicity (IARC 2013b), but that classification is based upon the 1987 monograph (Cogliano et al. 2011).

The US EPA classifies 2,4-D as "Group D-Not Classifiable as to Human Carcinogenicity" and the EU concluded: '*Proposed uses have no harmful effects on animal or human health; no evidence of carcinogenicity*.'

However, many epidemiological studies show an association between 2,4-D use and specific types of cancers, specifically soft tissue sarcoma and malignant lymphoma (for an overview see table 2 in von Stackelberg 2013). Some of these may be attributed to the dioxin/furan contamination (see e.g. Hardell 2008). Hardell et al. (1994) and many others reported higher cancer risk, when exposed to 2,4-D, but epidemiological studies have many weaknesses and results are often not significant, so that regulators cannot draw conclusions.

Some studies show that 2,4-D can be genotoxic, which is a sign that it could be mutagenic/carcinogenic, but other studies do not support this evidence. Amer & Aly (2001) showed for example a significant increase in the percentage of chromosome aberrations in mice bone-marrow and spermatocyte cells after oral administration of 2,4-D at 3,3 mg kg^{-1} bodyweight, but Knapp et al. (2003) did not find a significant increase in aberrant DNA rearrangements at doses from 3- 100 mg/kg/day in mice. Neither Mustonen et al. (1986) nor Garry et al. (2001) observed chromosome aberrations in exposed humans.

Korte & Jalal (1982) and Holland et al. (2002) found 2,4-D genotoxic *in vitro*. In the experiment by Holland et al. (2002), the commercial formulations showed a higher effect. Adjuvants or often secret inerts can either be toxic themselves or enhance certain effects. The results of Zeljezic & Garaj-Vrhovac (2004) which also show genotoxicity are therefore difficult to interpret, since the composition of formulation used is unknown.

Some evidence exists for toxic effects on reproduction. Japan has implemented the Globally Harmonized System (GHS) for the classification and labelling of pesticide and placed 2,4-D in Category 3: '*Suspected human reproductive toxicant'* because in a two generations fecundity study of rats , the survival rate of the offspring at doses which had an effect on parent animals was reduced, but no teratogenicity in the rat and rabbit was observed[46]. Schreinemachers (2003) associated use of 2,4-D and other related herbicides in four wheat producing states of the USA with birth malformations and other adverse perinatal outcomes. Arbuckle et al. (2001) report a moderate increased risk of early abortions for preconception exposures to phenoxy acetic acid herbicides such as 2,4-D [odds ratio (OR) = 1.5; 95% confidence interval (CI), 1.1–2.1].

A new study by Mosinger et al. (2013) relates exposure to 2,4-D and other phenoxy herbicides to male sterility and has potentially serious implications, because the effect cannot be shown in rodents and thus may be overseen by common risk assessment. Mosinger et al. (2013) showed that sperm formation without a functional specific taste receptor (T1R3) and its associated proteins is compromised, with malformed and immotile sperm. The taste receptors are expressed extra-orally in testis and sperm and seem to play a crucial role sperm development and maturation. Although the researchers did not conduct the experiment with 2,4-D, but used the antilipid medication clofibrate, they prooved in an earlier experiment that 2,4-D effectively inhibits the human taste receptors T1R2n& T1R3 (Maillet et al., 2009). The authors suggest that even low levels of chlorophenoxy compounds may lower sperm count and negatively affect human male fertility (Mosinger et al., 2013). The structural and functional similarity of clofibrates and 2,4-D was already shown by Vainio et al. (1983).

[46] See GHS Classification (ID134) in
http://www.safe.nite.go.jp/english/files/ghs_xls/classification_result_e(ID101-200).xls

Other effects

The European Union has classified 2,4-D and its salts and esters as a skin and respiratory sensitizer, and states that its use may cause allergic reactions such as contact dermatitis (EC 2008). Fukuyma et al (2009) also describe 2,4-D as a respiratory allergen, but did not find evidence for allergic reactions to skin.

This kind of classification can be a sign of immunotoxic properties. Faustini et al. (1996) support this hypothesis and conclude that exposure to commercial 2,4-dichlorophenoxyacetic acid (2,4-D) formulations may exert short term immunosuppressive effects. However more information on potential immunotoxic effects are not available.

In August 2013, the Australian authorization authority (APMVA) cancelled the use of several highly volatile 2,4-D esters[47], because "*the issue of persistence in the atmosphere, high volatility and potential to travel long distances in the environment along with their toxicity to non-target vegetation makes it very difficult to mitigate the risk from these compounds*" (APMVA 2013 p. 59). It was estimated that two of the esters (2,4-D EE and 2,4-D BE) might evaporate from the site of application and travel as far as 65 km through the air. However, in Europe these esters are not registered (APMVA 2013).

[47] http://www.apvma.gov.au/products/review/current/2_4_d.php

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Annex I: 2,4-D salts, ester, derivates

2,4-D salts, ester, derivates	Pesticide Compendium	BCPC Pesticide online Manual 6.0
2,4-D-dimethylammonium [2008-39-1]	Yes	Yes
2,4-D [94-75-7]	Yes	Yes
2,4-D-2-ethylhexyl [1928-43-4]	Yes	Yes
2,4-D-tris(2-hydroxypropyl)ammonium [18584-79-7]	Yes	Yes
2,4-D-isoctyl [25168-26-7]	Yes	Yes
2,4-D-isopropylammonium [5742-17-6]	Yes	Yes
2,4-D-butotyl [1929-73-3]	Yes	Yes
2,4-D-ethyl ester; 2,4-D EE [533-23-3]	Yes	Yes
2,4-D-sodium [2702-72-9]	Yes	Yes
2,4-D-butyl ester; 2,4-BE [94-80-4]	Yes	Yes
2,4-D-diolamine (2,4-D-diethanolammonium) [5742-19-8]	Yes	Yes
2,4-D-isopropyl [94-11-1]	Yes	Yes
2,4-D-isobutyl [1713-15-1]	Yes	Yes
2,4-D-trolamine [2569-01-9]	Yes	Yes
2,4-D-dodecylammonium [2212-54-6]	Yes	
2,4-D-ammonium [2307-55-3]	Yes	
2,4-D-2-butoxypropyl [1320-18-9]	Yes	
2,4-D-diethylammonium [20940-37-8]	Yes	
2,4-D-heptylammonium [37102-63-9]	Yes	
2,4-D-lithium [3766-27-6]	Yes	
2,4-D-meptyl [1917-97-1]	Yes	
2,4-D-methyl [1928-38-7]	Yes	
2,4-D-octyl [1928-44-5]	Yes	
2,4-D-pentyl [1917-92-6]	Yes	
2,4-D-propyl [1928-61-6]	Yes	
2,4-D-tefuryl [15146-99-3]	Yes	
2,4-D-tetradecylammonium [28685-18-9]	Yes	
2,4-D-triethylammonium [2646-78-8]	Yes	
Clacyfos [215655-76-8]	Yes	Yes

Annex II: The EU exclusion criteria

In November 2009 a new EU regulation for the authorization of pesticide active ingredients was published. Pesticides with certain toxicological properties are excluded from the authorization. Annex II (3.6.) gives the details:

'An active substance, safener or synergist shall only be approved if (…)

- it is not or has not to be classified, in accordance with the provisions of Regulation (EC) No 1272/2008, as mutagen category 1A or 1B.(…)
- is not or has not to be classified, in accordance with the provisions of Regulation (EC) No 1272/2008, as carcinogen category 1A or 1B (…).
- it is not or has not to be classified, in accordance with the provisions of Regulation (EC) No 1272/2008, as toxic for reproduction category 1A or 1B, (…)16
- it is not considered to have endocrine disrupting properties that may cause adverse effect in humans, (…).

The legislation does not provide a system to identify endocrine disruptors, but until criteria are developed 'substances that are or have to be classified, in accordance with the provisions of Regulation (EC) No 1272/2008, as carcinogenic category 2 and toxic for reproduction category 2, shall be considered to have endocrine disrupting properties.'

Annex III: GHS Classification for mutagenicity, carcinogenicity and reproductive and developmental toxins (CMR)

The classification for mutagenic chemical, carcinogenic chemical and reproductive and developmental toxins of Globally Harmonized System of Classification and Labelling of Chemicals (GHS) is divided into three categories: 1A, 1B and 2.

Category 1A, the highest category is based on human epidemiological studies and/or animal studies, 1B on positive results from in vivo tests in mammals. For Category 2 chemicals positive evidence must be obtained from in vivo and/or in vitro experiments.

The different categories are described as follows:

Mutagenicity

CATEGORY 1: Substances known to induce heritable mutations or to be regarded as if they induce heritable mutations in the germ cells of humans. Substances known to induce heritable mutations in the germ cells of humans.

Category 1A: The classification in Category 1A is based on positive evidence from human epidemiological studies.

Category 1B: Substances to be regarded as if they induce heritable mutations in the germ cells of humans.

The classification in Category 1B is based on:

- positive result(s) from in vivo heritable germ cell mutagenicity tests in mammals; or
- positive result(s) from in vivo somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells. It is possible to derive this supporting evidence from mutagenicity/genotoxicity tests in germ cells in vivo, or by demonstrating the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells; or
- positive results from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny; for example, an increase in the frequency of aneuploidy in sperm cells of exposed people.

Category 2: Substances which cause concern for humans owing to the possibility that they may induce heritable mutations in the germ cells of humans

- The classification in Category 2 is based on:
- positive evidence obtained from experiments in mammals and/or in some cases from in vitro experiments, obtained from:
- somatic cell mutagenicity tests in vivo, in mammals; or
- other in vivo somatic cell genotoxicity tests which are supported by positive results from in vitro mutagenicity assays.

Note: Substances which are positive in in vitro mammalian mutagenicity assays, and which also show chemical structure activity relationship to known germ cell mutagens, shall be considered for classification as Category 2 mutagens.

Carcinogenicity

CATEGORY 1: Known or presumed human carcinogens

A substance is classified in Category 1 for carcinogenicity on the basis of epidemiological and/or animal data. A substance may be further distinguished as:

Category 1A: Category 1A, known to have carcinogenic potential for humans, classification is largely based on human evidence, or

Category 1B: Category 1B, presumed to have carcinogenic potential for humans, classification is largely based on animal evidence.

The classification in Category 1A and 1B is based on strength of evidence together with additional considerations. Such evidence may be derived from:

- human studies that establish a causal relationship between human exposure to a substance and the development of cancer (known human carcinogen); or
- animal experiments for which there is sufficient (1) evidence to demonstrate animal carcinogenicity (presumed human carcinogen).
- In addition, on a case-by-case basis, scientific judgement may warrant a decision of presumed human carcinogenicity derived from studies showing limited evidence of carcinogenicity in humans together with limited evidence of carcinogenicity in experimental animals.

CATEGORY 2: Suspected human carcinogens

The placing of a substance in Category 2 is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations. Such evidence may be derived either from limited (1) evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies.

Reproductive toxicants

CATEGORY 1 Known or presumed human reproductive toxicant

Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B).

Category 1A Known human reproductive toxicant

The classification of a substance in Category 1A is largely based on evidence from humans.

Category 1B Presumed human reproductive toxicant

The classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific

consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate.

CATEGORY 2 Suspected human reproductive toxicant

Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification.

Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.