Environment & Health

Plastic Particles in Livestock Feed, Milk, Meat and Blood

A Pilot Study

Dr. I. van der Veen
Dr. L.M. van Mourik
M.J.M. van Velzen
Q.R. Groenewoud
Dr. H.A. Leslie

Final

Report EH22-01
29 April 2022

This report is released by: Prof. Dr. M. van Duursen

Head of the department of Environment & Health

mvd 16-05-2022

It was internally reviewed by: Prof. Dr. P.E.G. Leonards

Dr. H.A. Leslie has been involved in the design of the study and partly in its execution. After the termination of her employment at the VU, Dr Heather continued to contribute on personal titel to the preparation of the report.

This report was commissioned by: Plastic Soup Foundation | Contract T-O/2019-002

Dept. of Environment & Health
Faculty of Science
Vrije Universiteit Amsterdam
De Boelelaan 1085
1081 HV Amsterdam
The Netherlands
T +31 6 52 36 15 19
E pers@vu.nl

Plastic Soup Foundation
Sumatrakade 1537
1019 RS Amsterdam
The Netherlands

T +31 20 211 1348
E info@plasticsoupfoundation.org

Contents

Executive Summary

This pilot study sought to screen a variety of samples from livestock farms in the Netherlands for the presence of plastic particles. Studies of exposure to plastic particles on farms were lacking prior to this study. Detecting plastic particles in the small number of samples per sample type selected for this pilot study provided data that can be viewed as a starting point for larger scale studies aimed at elucidating the full scope of the distribution and range of concentrations of plastic particles in modern-day livestock feed and farm animals that consume it.

Samples collected for this pilot study

The sample set included four categories of samples:

- **Blood:** from pigs (n=12) and cows (n=12);
- **Milk:** from cows including hand-milked milk (n=5), tank milk (n=5), and packaged milk from supermarkets (n=15);
- **Feed**: feed pellets (n=9), fresh feed roughage[1] (n=5), and shredded supermarket feed (n=2);
- **Meat**: meat samples, all plastic-packaged, including filet and processed meat products (from both farms and supermarkets) (n=16).

Measurements of plastic particles

The samples in this study were analysed using a methodology and analytical technique that delivers the mass concentrations of different polymers commonly applied in plastic. Quality control included important steps such as monitoring and controlling any background plastic contamination potentially entering the samples during sample pre-treatment, through the use of calibration standards and injection standards, etc. The method had been previously validated for the analysis of human blood as described in (Leslie et al., 2022), however in our study we included the polymers of polyvinyl chloride (PVC-P).

First the particles were extracted from the samples. Next the polymers in the extracts were identified and quantified using instrumentation known as pyrolysis-gas chromatography/mass spectrometry (Pyr-GC/MS).

The size range of particles targeted for extraction and analysis were min. 700 nanometres in size and larger. The mass concentration quantified using the method is expressed as the mass per unit mass of the sample, e.g. µg polymer/g sample. The mass concentrations were determined for the sum of all extracted particles of a given polymer type in a given sample. Particles were not counted with this method. The measurement of polymer mass concentrations from plastic particles is analogous to the fine particulate mass concentrations reported in air pollution studies, e.g. PM10 or PM2.5, which are measured in micrograms per cubic metre.

The methodology applied did not measure the mass of additives (or any other small molecules) that may be present in the plastic. Polymers however make up the bulk of the mass of plastic materials and the polymer name e.g. polyethylene (PE), polystyrene (PS) is therefore commonly used to describe the plastic material as a whole.

The following high production volume polymers were targeted for analysis in all samples: polymers of polyvinyl chloride (PVC-P), poly(methyl methacrylate) (PMMA), polypropylene (PP), polymers of styrene (Styr-P), polyethylene (PE), and polyethylene terephthalate (PET). The method does not distinguish between the many different styrenic plastics, i.e. the homopolymer and copolymers of styrene, such as polystyrene, expanded polystyrene, acrylonitrile-butadiene-styrene etc., so polymers of styrene are reported here as a sum mass concentrations of all polymerized styrene materials present in the sample. The method does not distinguish between the many different PVC

[1] Hay-type feed, i.e. dried, slightly fermented vegetation

particles either, and so polymers of PVC are reported here as a sum mass concentrations of all polymerized PVC materials present in the sample.

Detected plastic particles concentrations

Analysis showed that particles of several types of plastic were present in the blood of both pigs and cows, in cows' milk both from the farms and from packaged milk from the supermarket, in farm animal feed pellets, and in packaged meat samples from three of the farms and from the supermarket.

Blood.

100% of the cow blood samples contained PVC-P (1.2-6.1 µg/g), PE (0.22-1.5 µg/g) and Styr-P (0.09-1.5 µg/g). PMMA and PET were not detected in any of the samples. PP (0.08-0.41 µg/g) was found in 33% of the samples.

PE (2.1->33 µg/g) and Styr-P (0.3->10 µg/g) were detected in 100% of the pig blood samples, PET (0.07-0.34 µg/g) in 42% and PP (0.16-0.37 µg/g) in 17%. In none of the samples PMMA was detected.

Milk.

Plastic particles were detected in 18 of 25 milk samples of all types. PP and Styr-P were not detected in any off the milk samples. PET in only 1 of the 25 samples (0.90 µg/g), PE in 2 samples (21 and 22 µg/g), PVC-P (6.1-13 µg/g) in 16% of the samples, and PMMA (0.11-1.2 µg/g) in 68% of the milk samples. Although some of the milk samples do contain one or more plastics types, a general answer cannot be given on how much plastic is present in Dutch cow's milk.

Meat.

7 of 8 beef samples contained at least one plastic type. PMMA, PP, and PET were not detected. Three samples contained PVC-P (53- >2600 µg/g), 3 samples contained Styr-P (77-200 µg/g) and 7 of 8 samples contained PE (150- >7700 µg/g).

5 of 8 pork meat samples contained at least one plastic type. PMMA, STYR-P and PET were not detected, PVC-P (127 µg/g) and PP (63 µg/g) each only in 1 sample and PE in 5 samples (88-690 µg/g).

Feed.

None of the fresh feed samples (n=5) contained detectable amounts of plastic particles. All other feed samples (n=12) contained at least PVC-P (339- >2600 µg/g), and PE (223- >2400 µg/g). 8 Samples also contained Styr-P (39-740 µg/g). PMMA, PP and PET were not detected.

Interpreting the measurements

Nowadays, it is well known that plastic particles are ubiquitous in marine, freshwater and terrestrial environments and in the human food chain. This study provided a first indication that plastic particles are also present at detectable concentrations on farms, in animal feed, in the animals themselves and in products from the farm (including processed, plastic-packaged products).

Plastic particles in feed pellets represents one of the possible exposure routes through which plastic particles reach the bodies of animals, alongside ingestion of water and respiration of air, (the latter two routes were outside the scope of this study and are currently included in other human exposure research initiatives). Uptake via the animal's skin is unlikely to occur unless the skin barrier is damaged and comes in contact with plastic particles.

The plastic particle concentrations measured in blood and hand-milked milk samples are indicative of the 'internal exposure' in the animals, i.e. the particles that had been absorbed in the animal's body. What we currently understand about plastic particles is that large particles are not absorbable across biological membranes. This means that any plastic particles detected in an animal's bloodstream itself are likely to be very tiny. Blood and hand-milked milk samples did not come into contact with plastic parts of milking equipment or plastic packaging, or the polymeric lining inside glass bottle caps (unlike supermarket milk and all meats packaged in plastic), and therefore are more indicative of the concentrations in the bloodstream and milk in the animal's body at the time of sampling.

The number of samples type in this pilot study was sufficient to demonstrate that it is possible to find plastic particles in blood of cows and pigs, cow's milk, livestock feed pellets, and meat products. A much larger number of samples should be measured in follow up studies to increase the understanding of the range of concentrations, frequency of detection in the animals and products, temporal and spatial variation in the concentrations, statistical differences between sample types etc. For a formal exposure assessment, hundreds of samples are typically required for achieving representative sampling for full statistical analysis. This was beyond the scope of this pilot study.

Outlook

The study was focused on measuring *exposure* to plastic particles, i.e. the concentrations that could be measured in the selected samples. Plastic sources, potential uptake routes and toxicological effects were outside the scope of this pilot study. No conclusions can be drawn regarding where all the plastic particles came from, or exactly how they entered the animals' bodies or feed, milk and meat. This work does provide a starting point for developing new hypotheses to test. Also, until further research is completed it remains unknown if there are any potential toxicological risks of these findings. Exposure data are an essential element of risk assessment, as they give information on the real-world plastic particle concentrations that are present. These concentrations can then be compared to the threshold concentrations above which adverse effects on populations are expected, as determined in laboratory toxicity tests.

Conclusion

These pilot study data indicate that animals are able to absorb at least some of the plastic particles they are exposed to in their living environment (i.e. via feed, air and/or water), This results in detectable concentrations of plastic in the blood of pigs and cows. Some milk and meat products also contain plastic, though it has not yet been investigated if this is the result of plastic absorption into the animal body from feed, water and/or air, or the result of processing and/or packaging of the milk and meat, or all of the above. This study reports on the measured concentrations though not on how each of the plastic types got there. However ingestion is an uptake route that is suspected to play a major role in determining plastic doses and internal exposure. No conclusion can be made whether the concentrations observed in this study are safe or not safe until toxicological data is collected. This pilot study should act as an impetus to further explore the full scope of exposure and any risks that may be associated with it. The production of plastic-free feed for animals may be one of the ways to improve the plastic particle exposure scenario for livestock.

1 Introduction

The Netherlands is home to millions of livestock animals for human consumption. There are currently around 3.7 million cows and 11.5 million pigs (CBS, 2021a, b). About 1 million sheep are kept, as well as about 0.33 million goats for milking (CBS, 2016). These farm animals may be exposed to plastic particles via their feed and other exposure routes such as water or air, of which dairy (and/or meat) products ends up as human consumption. However, data to confirm this are missing.

Exposure through freshwater and air is likely as they contain plastic particles (Dris et al., 2017; Leslie et al., 2017). Plastic potentially present in the soil of grazing meadows may be inadvertently consumed by grazers who are known to consume soil along with the vegetation. Dermal uptake is not expected unless there is damage to the skin and contact with plastic (e.g. inflamed udders in contact with plastic milking robots).

Exposure through feed is even more likely. The Netherlands produces about 35 million tonnes of animal feed annually: 16 million tonnes of roughage animal feed (esp. grass, maize) and 19 million tonnes of other feed (mixtures etc.) (BuRO, 2019). The feed for farm animals is highly diverse, seasonal, age-specific and, of course, species-specific. For instance, young calves receive powder milk, young pigs receive pellets. Older animals receive a combination of many different feed sources including 'roughage' (grass, hay, in Dutch: '*kuil*') and supplements' (in Dutch: '*krachtvoer*'). In addition, food for human consumption is regularly part of the farm animal diet, either excess pre-consumer vegetables such as potatoes, beets, or scraps thereof leftover from processing (e.g. potato skins). There is also pre-consumer supermarket waste, esp. bread, pastries and sweets that are no longer fit for the human-consumption market because the expiry date has been passed. There are European guidelines and legislation for this practice. These types of feed products are often locally sourced. The Netherlands also imports animal feed, e.g. soy products.

In 2018, the Food Safety Authority of the Netherlands (NVWA) published a notice (NVWA, 2018) that livestock farmers in the Netherlands are required to remove all plastic packaging from the feed associated with the feed bales before offering it to the animals. This occurred after inspectors from the NVWA noticed that poultry, cows, sheep, goats and horses were receiving feed that still had plastic fully or partially attached to the feed, sometimes with the intention to keep the feed clean. In the notice, the NVWA states that plastic is a harmful material that carries risks for the wellbeing and health of the animals, which cannot digest it. The NVWA notice further states that it is forbidden to give plastic to the livestock because this way the animals may consume some plastic along with their food. Despite the notice, the plastic wrapping of feed bales are still not always removed for various reasons, e.g. keeping the roughage moist or keeping air out. Sometimes there are holes made in the plastic feed bale packaging for animals to pick out the grass from the packaging. Especially nonselective eaters such as cows may ingest plastic via this route.

If animals are being fed plastic particles, questions arise about what this means for animal exposure to micro and/or nanoplastics, animal welfare, animal dignity, meat and dairy product quality, and ultimately nano- and microplastic exposure in humans via consumption of food produced with these animals. Once inside the animals' bodies, a fraction of the plastic particles may potentially translocate across epithelial membranes into the bloodstream. The blood irrigates all of the organs and is pumped through the udders and mammary glands of cows and other mammals to produce milk. It is plausible that plastic particles are present in the food chain of farm animals, which ends as dairy and/or meat products for human consumption. However, data are needed to confirm whether plastic particles are present in the feed, the bloodstream and the milk of farm animals in the Netherlands. In this study, the main aim was to perform a pilot project to explore aspects of the exposure of farm animals in the Netherlands to plastic particles:

First research question: Do Dutch livestock animals (cows, pigs) have plastic particles in their blood?

Second research question: How much plastic is present in Dutch cow's milk?

Third research question: Are plastic particles present in beef and pork?

Fourth research question: Are Dutch livestock animals exposed to plastic particles via their feed?

Cows and pigs were chosen as sentinel species[2]. They represent two major livestock taxa in the Netherlands. Together they have links to humans via consumption of meat, and for cows, also dairy. There are also some suspected issues with plastic and the feed of these livestock animal types. Pigs are omnivores and candidates for mixed feed including supermarket waste food. Both cows and pigs in the Netherlands may be fed with supermarket pre-consumer food waste, as long as it does not contain animal products.

The research questions are designed to generate novel data and shed light on a unique area of 'micro- and nanoplastics' research (farm animal exposures) that has not received sufficient attention from the research community to date. The pilot studies serve to generate more questions about animal welfare and toxicological consequences for humans and animals, and generate more public awareness and public interest and political will to see follow-up questions answered in future studies. The studies do not produce comprehensive data sets, rather they serve to signal where risks may lie and in which directions future research is needed.

[2] Sentinel species are organisms that are investigated in the context of pollution studies in order to provide an early warning of exposure and risk to humans.

2 Materials and Methods

2.1 Study overview

This pilot study consists of four parts:

<u>Part 1</u>: Determining plastic particles in blood of livestock animals

In this first part we analysed plastic particles in blood of cows and pigs of Dutch farms. Blood as a sample matrix is central to this study, and there are several reasons for including farm animal blood analysis. Firstly, plastic particles concentrations in blood indicates the ‘internal’ exposure of cows and pigs, integrating all potential exposure routes (ingestion and inhalation, i.e. via feed, water, and air). Secondly, blood bathes all organs and muscle tissue of the animal, and the bloodstream is the route by which plastic particles can travel inside the body to potential sites of toxic action. Thirdly, blood is present in the meat for human consumption of these animals so it also makes the link to human exposure. In addition, blood plays a central role in the transfer of ingested or inhaled plastic particles in the body of the cow to the milk, and ultimately to human consumers of dairy products. All milk precursors are supplied by the large volumes of blood flowing to the udders via major arteries, for use in milk production (ca. 500 litre of blood flows through the udders for every 1 litre of milk produced, cf. (FAO, 2021; Cortes, ESA manual). We hypothesized that for at least some of the of blood samples, we would be able to report at least one polymer type from plastic present above analytical detection limits.

<u>Part 2</u>: Determining plastic particles in cow’s milk

Milk production is inextricably connected to environmental conditions – milk as environmental contaminant matrix is common in toxicological studies. We determined plastic particles in cow’s milk. Concentrations of plastic particles in milk is relevant to human exposure, but also to calves’ exposure via their food consumption. In addition, concentrations of plastic particles measured in milk could also provide proof of ‘internal’ exposure in cows (provided the milk is sampled without contact with plastic). A previous study of supermarket packaged milk reported that microplastics were present in cow’s milk (Diaz-Basantes et al., 2020). The processing and packaging of the milk were among the sources of the plastic contamination so no conclusion could be made regarding what came from the cows’ bodies directly.

To make a distinction between the milk that may have been contaminated with plastic before and after leaving the cow’s body, this study included not only tank milk but also hand drawn milk and packaged supermarket milk.

Hand drawn milk does not make use of plastic (PVC) milking robots on the udders and plastic tubing to bring the milk to the tank. This sampling method reflects most closely the plastic content that was transferred from blood to milk inside the cow’s body.

Tank milk comes from the tank containing milk that was acquired via plastic-containing milking robots. Milk from the milking robots is transferred through pipes containing plastic to a tank that may have been cleaned with plastic instruments and materials. The farm-based plastic background contamination is therefore included in the tank milk samples.

Supermarket packaged milk is tank milk that has been further processed, packaged and transported. The packaging of milk varies from glass to plastic bottles, tetra pack, or plastic (e.g. polyethylene) coatings inside milk carton packaging. Through the packaging, milk purchased in the supermarket has potentially been in contact with multiple plastic types, prior to consumption and sampling.

For the milk study, a small number of milk samples was analysed: 5 from tank milk, 5 from hand drawn milk, 5 from packaged supermarket milk. These different types of milk sample (n=5 each) cover different potential sources of contamination by plastic, from cow through processing to packaging. Concentrations measured across the whole set of milk samples can give a glimpse of Dutch milk in general. If the differences are large between the milk type groups, it would inform hypotheses regarding at which point in the pathway from cow to glass the plastic contamination may be introduced.

Part 3: Determining plastic particles in beef and pork

If plastic particles are present in the blood of farm animals, the next step is to search for plastic particles in the tissues bathed by the animals' blood. Pork and beef are such tissues and contain animal blood. The blood surrounds the cells that make up the muscle or organ tissue, which is used to make the meat product. We hypothesized that the plastic particles could be detected in meat of cows and pigs with a frequency similar to that of the blood sampled from cows and pigs. Just like blood, concentrations of plastic particles in meat indicate 'internal' exposure of cows and pigs, integrating all potential exposure routes (food, water, air). In addition, concentrations of plastic particles in meat make the link with human consumption via the food chain. Last but not least, plastic polymer presence in muscle or organ tissue can be considered first step towards a baseline for exposure assessment that can be used in animal health risk assessment.

Part 4: Determining plastic particles in feed

If plastic particles are detected in farm animal blood, milk and meat, it is clear that this 'internal dose' exposure results from exposure to plastic particles via sources outside the body in the farm environment. Investigating samples that represent external exposure routes gives insight into the reasons why farm animals might have observable plastic polymer body residues. While 'internal dose' gives information about plastic particles near the target site for toxic action and potential concentrations in food for human consumption, 'external' dose exposure estimates give more direction to potential mitigation options. For example, feed is one of the major potential sources of plastic particles. Looking into the external sources of exposure aids in the designing of potential mitigation measures that address exposure prevention. Animal feed was chosen as a source of animal exposure, also because it would potentially expose an unintentional consequence of the circular economy should too much plastic be demonstrated to be infiltrating the animal feed cycles.

2.2 Sampling

Sampling of all matrices (blood, milk, meat and feed) from livestock farms was carried out by a qualified veterinary doctor. Samples related to cows (blood, meat, milk, and feed) originated from 6 different livestock farms (Farms A-F). More information on the farms and cows is given in Table 1.

The samples related to pigs (blood, meat, and feed) originated from one of the farms which also delivered samples for cows (Farm C) and one other farm (Farm G) (See Table 2). Additional pig feed samples from another pig farm were later sampled by the veterinarian.

A sampling protocol was prepared by the VUA and special effort was made to reduce background contamination during sampling. The sampling containers were prepared (cleaned) by the VUA and delivered to the veterinarian for sampling. Milk samples were taken at the farms using precleaned containers provided by the VUA. Sampling took place on 30 June and 1 July 2021 (See tables 2-7).

Three farmers provided a meat sample from their stocks. All other meat samples were acquired from supermarkets.

Supermarket milk samples from different brands were purchased by VUA staff in September 2021. Supermarket and butchery meat samples were purchased by VUA staff on 30 November 2021.

Table 1 Information about cows and farms. Farm F provided powdered milk sample feed.

Farm	Cow number	Day of birth	Type	Organic (yes/no)	Only indoor/ also outside
A	A-1	17/03/2017		No	indoor
A	A-2	24/11/2017		No	indoor
A	A-3	25/12/2018		No	indoor
B	B-1	22/01/2015		No	indoor
B	B-2	11/02/2009		No	indoor
B	B-3	30/07/2017		No	indoor
C	C-1	07/02/2018	Jersey	No	also outside
C	C-2	11/08/2016	Jersey	No	also outside
C	C-3	28/12/2017	Jersey	No	also outside
D	D-1	13/09/2017		Yes	also outside
D	D-2	14/09/2017		Yes	also outside
D	D-3	07/10/2011		Yes	also outside
E	E-1	13/12/2012		yes	also outside
E	E-2	13/05/2017		Yes	also outside
E	E-3	13/09/2014		Yes	also outside
F*				Yes/No	n.a.

n.a. not available

* Farm F provided powdered milk and sample feed.

Table 2 Information about pigs and farms

Farm	Type	Organic (yes/no)	Only indoor/ also outside
C	Porker	No	Indoor
G	Porker	No	indoor

2.2.1 Blood samples

VUA received 24 blood samples from cows (n=12) and pigs (n=12) (See Table 3) for the analyses of microplastics. The samples were stored at -20 °C until extraction and analysis.

Table 3. Information on cow and pig blood samples

LIMS code[a]	Sample Type	Farm	Animal Number	Animal Age	Sampling date	Sampling time
21/1307	Cow blood	A	A-2	3.6 years	01/07/2021	8.00-9.30
21/1310	Cow blood	A	A-3	2.5 years	01/07/2021	8.00-9.30
21/1314	Cow blood	B	B-1	6.4 years	01/07/2021	10.00-11.00
21/1317	Cow blood	B	B-2	12.4 years	01/07/2021	10.00-11.00
21/1319	Cow blood	B	B-3	3.9 years	01/07/2021	10.00-11.00
21/1322	Cow blood	C	C-1	3.4 years	01/07/2021	11.20-12.00
21/1326	Cow blood	C	C-2	4.9 years	01/07/2021	11.20-12.00
21/1330	Cow blood	C	C-3	3.5 years	01/07/2021	11.20-12.00
21/1334	Cow blood	D	D-1	3.8 years	01/07/2021	14.00-15.00
21/1338	Cow blood	D	D-2	9.7 years	01/07/2021	14.00-15.00
21/1342	Cow blood	E	E-2	4.1 years	01/07/2021	15.30-16.30
21/1344	Cow blood	E	E-3	6.8 years	01/07/2021	15.30-16.30
21/1347	Pig blood	C	C-V1	3.5 months	01/07/2021	12.00-13.00
21/1349	Pig blood	C	C-V3	3.5 months	01/07/2021	12.00-13.00
21/1350	Pig blood	C	C-V4	3.5 months	01/07/2021	12.00-13.00
21/1351	Pig blood	C	C-V5	3.5 months	01/07/2021	12.00-13.00
21/1922	Pig blood	G	pig 1	3 months	05/07/2021	n.a.
21/1923	Pig blood	G	pig 2	3 months	05/07/2021	n.a.
21/1924	Pig blood	G	pig 3	3 months	05/07/2021	n.a.
21/1925	Pig blood	G	pig 4	3 months	05/07/2021	n.a.
21/1926	Pig blood	G	pig 5	3 months	05/07/2021	n.a.
21/1927	Pig blood	G	pig 6	3 months	05/07/2021	n.a.
21/2762	Pig blood	G	pig 7	3 months	04/11/2021	n.a.
21/2855	Pig blood	G	pig 8	3 months	04/11/2021	n.a.

n.a. not available

[a] LIMS is VUA sample code

2.2.2 Cow's milk samples

Fifteen milk samples of different types (skimmed, semi-skimmed, UHT treated, etc.) were purchased from supermarkets, and 11 milk samples were sampled at livestock farms. More information on the type of samples, the containers the samples were purchased in, and the sampling or purchase dates are given in Table 4.

Table 4. Information on cow's milk samples

LIMS code[a]	Sample Type	Farm/ store	Animal Number	Sample date/ purchase date	Best before...	Container
21/2494	skimmed	Supermarket		05/10/2021	10/10/2021	refrigerated carton
21/2495	skimmed	Supermarket		05/10/2021	11/10/2021	refrigerated carton
21/2497	skimmed, UHT treated	Supermarket		05/10/2021	14/03/2022	shelf-stable carton
21/2498	skimmed, UHT treated	Supermarket		05/10/2021	3/03/2022	shelf-stable carton
21/2484	semi-skimmed, UHT treated	Supermarket		05/10/2021	12/10/2021	plastic
21/2496	lactose-free semi-skimmed, UHT treated	Supermarket		05/10/2021	3/03/2022	shelf-stable carton
21/2485	semi-skimmed milk	Supermarket		05/10/2021	17/01/2022	plastic
21/2486	semi-skimmed milk	Supermarket		05/10/2021	12/10/2021	plastic
21/2487	semi-skimmed milk	Supermarket		05/10/2021	10/10/2021	plastic
21/2488	semi-skimmed milk	Supermarket		05/10/2021	12/10/2021	plastic
21/2489	semi-skimmed milk	Supermarket		05/10/2021	8/10/2021	glass with cap
21/2490	semi-skimmed milk	Supermarket		05/10/2021	8/10/2021	glass with cap
21/2491	semi-skimmed milk	Supermarket		05/10/2021	12/10/2021	glass with cap
21/2492	semi-skimmed milk	Supermarket		05/10/2021	12/10/2021	glass with cap
21/2493	full	Supermarket		05/10/2021	5/10/2021	glass with cap
21/1355	Milked by hand	Farm A	A-992	01/07/2021		
21/1357	Milked by hand	Farm B	B-9876	01/07/2021		
21/1358	Milked by hand	Farm C	C-8441	01/07/2021		
21/1359	Milked by hand	Farm D	D-1060	01/07/2021		
21/1360	Milked by hand	Farm E	E-0174	01/07/2021		
21/1361	Tank	Farm A		30/06/2021		
21/1362	Tank	Farm B		01/07/2021		
21/1363	Tank	Farm D		01/07/2021		
21/1364	Tank	Farm E		01/07/2021		
21/1365	Tank	Farm F		01/07/2021		
21/1366	Powder for calves	Farm F		01/07/2021		

[a] LIMS is VUA sample code

2.2.3 Beef and pork samples

Two beef samples and one pork sample from three different livestock farms were delivered to the VUA. In addition, different types of beef and pork samples were purchased at a supermarket (beef n=3, pork n=4), and a butchery (beef n=3, pork n=3). More information about the meat samples is given in Table 5 (beef) and Table 6 (pork). All meat samples were stored at -20 °C until extraction and analysis.

Table 5. Information on beef samples

LIMS code[a]	Sample Type	Farm/ store	Sample date/ purchase date
21/1387	beef	Farm B	1/7/2021
21/1389	beef	Farm D	1/7/2021
21/2813	round steak	Supermarket	30/11/2021
21/2814	organic burger	Supermarket	30/11/2021
21/2815	organic steak	Supermarket	30/11/2021
21/2821	round steak	Butchery	30/11/2021
21/2819	fine ribs	Butchery	30/11/2021
21/2820	roasts	Butchery	30/11/2021

[a] LIMS is VUA sample code

Table 6. Information on pork samples

LIMS code[a]	Sample Type	Farm/ store	Sample date/ purchase date
21/1388	pork	Farm C	1/7/2021
21/2811	ham steak	Supermarket	30/11/2021
21/2810	tenderloin	Supermarket	30/11/2021
21/2809	fricandeau	Supermarket	30/11/2021
21/2812	fillet	Supermarket	30/11/2021
21/2816	ham steak	Butchery	30/11/2021
21/2818	tenderloin	Butchery	30/11/2021
21/2817	chop	Butchery	30/11/2021

[a] LIMS is VUA sample code

2.2.4 Livestock Feed samples

Sixteen different types of feed from livestock farms were delivered to the VUA (See Table 7). All feed samples were stored at -20 °C until extraction and analysis.

Table 7. Information on feed samples

LIMS code[a]	Sample Type	Farm	Sample date
21/1375	Cow pellets	A	01/07/2021
21/1378	Cow pellets	B	01/07/2021
21/2794	Cow pellets	C	01/07/2021
21/1383	Cow pellets	D	01/07/2021
21/1385	Cow pellets	E	01/07/2021
21/1381	Pig pellets	C	05/07/2021
21/2795	Pig pellets	G	22/11/2021
21/2796	Pig pellets	G	22/11/2021
21/2797	Pig pellets	G	22/11/2021
21/1376	Fresh feed	A	01/07/2021
21/1380	Fresh feed	B	01/07/2021
21/1382	Fresh feed	D	01/07/2021
21/2798	Fresh feed	D	01/07/2021
21/1384	Fresh feed	E	01/07/2021
21/2856	Shredded feed	F	1/12/2021
21/2857	Shredded feed (organic)	F.	1/12/2021

n.a. not available

[a] LIMS is VUA sample code

2.3 Sample handling and preparation

2.3.1 Blood samples

The blood samples were thawed at room temperature, and prior to extraction homogenized on a roller bank for one hour. 2 mL of blood sample was weighed into a pre-rinsed scintillation vial. After adding 15 mL of TRIS-HCl buffer (400 mM Trizbase, pH 8 (HCl), 0.5% Sodium Dodecyl Sulfate (SDS)) to the samples, the samples were heated in a water bath for 1 hour at 60˚C. 100 µL freshly prepared Proteinase K, and 1 mL 5 mM $CaCl_2$ were added consecutively, before conditioning in a water bath at 50˚C for at least 2 hours. The samples were shaken on a shaking table at max 150 times/min for 20 minutes, before heating in a water bath for 20 minutes at 60˚C.

Samples were filtrated over a clean glass filter (25 mm GF/F filter Whatman) on a special glass setup with vacuum filtration. 1 mL of filtrated hydrogen peroxide (30%, Merck) was added to the filter, before rinsing of the filter with 15 mL filtrated Milli-Q. The center of the filter was cut out with a cleaned puncher, and added into a clean pyrolysis cup. 10 µL tetramethylammonium-hydroxide (TMAH) (25 % wt in methanol) and 25 µL internal standard (Polystyrene-d5 (0.15 µg)) were added to the filter. Prior to analyses with pyrolysis-gas chromatography/mass spectrometry (Pyr-GC/MS), the filters were dried overnight in an oven at 40˚C.

Four of the pig blood samples (21/1922, 21/1925, 21/2762 and 21/2855) were only partly extracted over the filter due to clogging of the filter.

2.3.2 Cow's milk samples

The milk samples were thawed at room temperature, and prior to extraction homogenized on a roller bank for one hour. Approximately 1 mL of milk sample was weighed into a pre-rinsed scintillation vial. After adding 15 mL of TRIS-HCl buffer (400 mM Trizbase, pH 8 (HCl), 0.5% Sodium Dodecyl Sulfate (SDS)) to the samples, the samples were heated in a water bath for 1 hour at 60˚C. 100 µL freshly prepared Proteinase K, and 1 mL 5 mM $CaCl_2$ were added consecutively, before conditioning in a water bath at 50˚C for at least 2 hours. The samples were shaken on a shaking table at max 150 times/min for 20 minutes, before heating in a water bath for 20 minutes at 60˚C. Samples were filtrated over a clean glass filter (25 mm GF/F filter Whatman) on a special glass setup with vacuum filtration. 10 mL of filtrated hydrogen peroxide (30%, Merck) was added to the filter, followed by 2 times 0.5 mL sulfuric acid, before rinsing of the filter with 15 mL filtrated Milli-Q. The center of the filter was cut out with a cleaned puncher, and added into a clean pyrolysis cup. 10 µL TMAH (25 % wt in methanol) and 25 µL internal standard polystyrene-d5 (0.15 µg abs. in cup) were added to the filter. Prior to analyses with Pyr-GC/MS, the filters were dried overnight in an oven at 40˚C.

2.3.3 Beef and pork samples

Before sample intake, the outer parts of the meat were cut off, because those could be contaminated with microplastics from the plastic packaging. Approximately 20 grams of meat was weighed into a cleaned sample jar to be dried by freeze drying at -20˚C. After freeze drying, the sample was homogenized in de sample jar by using a spoon. 3 grams of freeze-dried meat was cleaned by accelerated solvent extraction (ASE) with methanol. Plastics were extracted from the sample by ASE with tetrahydrofuran. After extraction, an aliquot of 25 µL (from approx. 40 mL) extract was transferred into a clean pyrolysis cup. After evaporation of the solvent in an oven at 40˚C, 75 µL hydrogen peroxide (30%) was added to the cup. The sample extract was conditioned at room temperature for 2 hours to allow the hydrogen peroxide to oxidize the matrix. The hydrogen peroxide was evaporated overnight at 105˚C. Prior to analyses with Pyr-GC/MS, 10 µL internal standard 4-Fluorostyrene (0.24 µg abs. in cup) was added. The solvent was evaporated in the cup in an oven at 40˚C.

2.3.4 Livestock Feed samples

Because of the high amount of water present in the two shredded feed samples (21/2856 and 21/2857), these samples were freeze-dried prior to sample intake and extraction. The freeze-dried shredded feed samples, and the pellet samples were finely ground and homogenized by using a mortar and pestle. The fresh feed samples were cut in small pieces (<1 cm) by using a pair of scissors. Approx. 3 grams of the freeze-dried samples, 10 grams of the homogenized pellets and 3 grams of the fresh feed samples were cleaned by ASE with methanol. Plastics were extracted from the samples by ASE with tetrahydrofuran. After extraction, an aliquot of 25 µL (from approx. 40 mL) extract was transferred into a clean pyrolysis cup. After evaporation of the solvent in an oven at 40˚C, 10 µL internal standard 4-Fluorostyrene (0.24 µg abs. in cup) and 10 µL TMAH (25 % wt in methanol) were prior to further analyses with Pyr-GC/MS.

2.4 Analysis

All samples were analysed for their content of poly(methyl methacrylate) (PMMA), polypropylene (PP), polymers of styrene (Styr-P), polyethylene (PE), and polyethylene terephthalate (PET), according to a previously described and validated method (Leslie et al., 2022). In addition the polymers of polyvinyl chloride (PVC-P) were analysed.

Analysis was performed using the multishot pyrolysis unit EGA/PY-3030D (Frontier Laboratories, Saikon, Japan) in “double shot” mode. First, the sample was placed in the pyrolyzer unit at 100 °C, which was then heated to 300 °C at a rate of 50 °C/min. After the sample was retracted, the GC/MS measurement started for any volatile compounds present on the filter, as they thermally desorb between 100 and 300 °C. The GC/MS (Agilent 6890 GC and 5975C MS, Santa Clara CA, USA) was equipped with a Ultra Alloy-5 column (30 m x 0.25 mm x 0.25 µm, Frontier Laboratories, Saikon, Japan). Measurements were done in Full scan mode (m/z 33 – 500) and in split mode (1:50 split ratio for feed samples; 1:10 split ratio for all other samples). The compounds were trapped on the GC column. The column was programmed from 40 °C (2 min.) at a rate of 20 °C/min to 360°C, and then 2 min. at 360 °C, resulting in a total run time of 20 min. After the thermal desorption step, the pyrolyzer was heated to 600 °C and the filter was again introduced (1 min.) for the next measurement (pyrolysis). The column was programmed from 40 °C (2 min.) at a rate of 40 °C/min to 360 °C (2 min.), resulting in a total run time of 20 min.
The compounds that are desorbed in the first run (‘shot’) are molecules that are volatilized at 300 °C and can include unpolymerized monomers, additives and other sorbed chemicals. Any monomers (e.g. benzene, styrene) potentially present in this run were not used in determining concentrations of plastic particles, except for PET where the derivatization product already forms at 300 °C and the results from both the first and second shots were combined. The pyrolysis second ‘shot’ chromatograms were used for determination of the other polymer concentrations associated with polymers.

Data processing was performed using the Agilent Masshunter software, and the settings as shown in Table 8.

Table 8. GC/MS data processing information for plastics

Polymer	Product	Mass (m/z)	RT (min)
PVC-P	Benzene	78, 77, 52	2.879
	1-Methylindene*	130, 129, 115	8.027
	1-Methylnaphthalate	142, 141, 115	9.288
PMMA	Methylmethacrylate	100, 69, 85	3.352
PP	Dimethylheptene	126, 70, 83	4.960
Styr-P	Styrene*	104, 103, 78	5.536
	Styrene trimer	91, 312, 117	15.374
PE	1-Decene	83, 97, 111	6.548
	1-Undecene*	83, 70, 97	7.441
	1-Tetradecene	83, 97, 69	9.692
PET**	Dimethylterephthalate (TMAH)	163, 194, 135	10.502
PET***	Benzophenone	105, 94, 77	6.705
PS-d5****	Styrene d5	109, 108, 82	5.516
4-Fluorostyrene*****	4-Fluorostyrene	122, 121, 96	3.907

* Quantifier

** TMAH is used here to analyze PET. These masses were used for analyzing blood, milk and livestock feed samples.

*** Benzophenone is used for quantifying PET without adding TMAH. This fragment is used for analyzing pork and beef meat samples.

**** Styrene d5 is used as an internal standard for analysis of blood and milk samples.

***** 4-Fluorostyrene is used as an internal standard for analysis of livestock feed samples, pork and beef meat samples.

A value given between limit of detection (LOD) and limit of quantification (LOQ) means that the analyte was present but the concentration was too low to quantify as accurately as values >LOQ. Results were still detected but at values between the LOD and LOQ.

2.5 Quality assurance and quality control (QA/QC)

To avoid blank contamination precautions have been taken, and in addition blank control samples have been analysed together with the samples, as described in paragraph 2.5.1 t/m 2.5.4

2.5.1 Blood samples

Procedural blanks

Six procedural blank samples were extracted and analysed in the same series, and with the same procedure as the cow, and pig blood samples (See 2.3.1). The blank samples consisted out of pre-rinsed scintillation vial, to which the 15 ml TRIS-HCl buffer was added.

Three additional blank samples were extracted and analysed in the same series as four pig blood samples, which were only partly extracted over the filter due to clogging of the filter (21/1922, 21/1925, 21/2762 and 21/2855).

Needles and collection tubes

For sampling of the cow- and pig blood, needles and collection tubes were used. 3 blank needles (needle b1- needle b3) and 3 blank tubes (tube b1- tube b3) were extracted and analysed.

9 ml of filtered MiliQ water was passed through the needles, of which a 2 ml aliquot was filtered and analysed.

The tubes with 9 mL of filtered MiliQ water were placed on a roller bank for one hour. An aliquot of 2 mL was filtered and analysed.

2.5.2 Cow's milk samples

Procedural blanks

Five procedural blank samples were extracted and analysed in the same series, and with the same procedure as the milk samples (See 2.3.2). The blank samples consisted out of pre-rinsed scintillation vial, to which the 15 ml TRIS-HCl buffer was added.

Prerinsed samples containers

Prior to sampling of the milk samples at the farms, the sample containers were rinsed three times with MiliQ water, and dried upside down covered by aluminium foil by natural evaporation by air.

2.5.3 Beef and pork samples

Procedural blanks

Two procedural blank samples were extracted and analysed in the same series, and with the same procedure as the meat samples (See 2.3.3). The blank samples consisted out of extra pure sea sand (nr.14808-60-7, Sigma-Aldrich) which was cleaned by heating at 600°C for 4 hours.

Sample package

The avoid blank contamination of the pork and meat samples which were purchased at the supermarket and the butchery from the packaging material, only the inner side of the meat, which had not been in contact with the packaging material was analysed

2.5.4 Livestock Feed samples

Seven procedural blank samples were extracted and analysed in the same series, and with the same procedure as the feed samples (See 2.3.4). The blank samples consisted out of extra pure sea sand (nr.14808-60-7, Sigma-Aldrich) which was cleaned by heating at 600°C for 4 hours. For four of those blank samples 3 gram sea sand was weighted as representative amount for the fresh feed samples. Ten gram sea sand was used for the other four blank samples as representative

3 Results and Discussion

3.1 Blood samples

Five of the six polymers were detected in animal blood: PVC-P, PP, Styr-P, PE, and PET (see Table 9, Figures 1 and 2, Appendices A and B). PMMA was the only target analyte that was not encountered in any of the blood samples. PET was detected in pigs only, not in cows.

It should be noted that four of the twelve pig blood samples (21/1922, 21/1925, 21/2762 and 21/2855) were only partly extracted due to clogging of the filter. Since it cannot be guaranteed that the extracts were homogeneous, the calculated concentrations should be considered to be indicative values. In addition, three pig blood samples (21/1925, 21/2762, and 21/2855) had very high concentrations of PVC-P and PE and in two cases Styr-P. The values were calculated to be in the range above the highest calibration standards, therefore these values were reported as ≥ the value of the highest calibration standard and must be considered to be indicative (See Table 9).

PMMA was not detected in any of the blood samples, and PET was only detected in 5 of the 12 pig blood samples (0.07-0.34 µg/g). PP was detected in 4 of the 12 cow blood samples (0.08-0.40 µg/g), and in 2 of the 12 pig blood samples (0.16-0.37). The other three plastics, PVC-P, Styr-P and PE were detected in all blood samples. The concentrations of all those three plastics were higher in the pig blood samples (PVC-P: 1.7-17 µg/g, Styr-P: 0.3-10 µg/g; PE 2.1-33 µg/g) than in cow blood samples (PVC-P: 1.2 - 6.1 µg/g; Styr-P: 0.09-1.5 µg/g; PE 0.22-2.9 µg/g).

The pigs from farm C were all 3.5 months old. The pigs of farm G were a bit younger (3 months). The pigs were fed with milk until they were 3 a 4 weeks old. No difference in polymer types and concentrations is observed related to the age of the pigs. All pig blood samples were taken from pigs of regular farms.

Cow blood samples were taken from cows at regular farms (n=8) as well as from organic farms (n=4). There is no observed difference in detected plastic types and concentrations in the blood samples originating from the regular and the organic farms (See Figure 1). No relation could be found between the age of the cows and the concentration of plastic particles either.

µ

It was emphasized a priori that if e.g. 10% of the animals in the study are shown to have plastic particles in their blood >LOD (limit of detection), it could be considered a signal of plastic polymer exposure in the livestock population. Notably in the blood samples of all tested animal at least three different types of plastic particle concentrations were detected >LOD.
With these results, the first research question of whether Dutch livestock animals (cows, pigs) have plastic particles in their blood, was answered positively.

Table 9. Concentrations (µg/g) of 6 polymers analysed in cow and pig blood samples, per polymer type.

LIMS code[a]	Sample Type	Farm	Farm Organic (yes/no)	Animal Number	PVC-P	PMMA	PP	Styr-P	PE	PET
21/1307	Cow blood	A	No	A-2	2.9	<0.40	<0.08	0.56	1.4	<0.08
21/1310	Cow blood	A	No	A-3	6.1	<0.40	<0.07	0.84	2.9	<0.07
21/1314	Cow blood	B	No	B-1	1.2	<0.40	<0.07	*0.09	0.26	<0.07
21/1317	Cow blood	B	No	B-2	2.0	<0.40	<0.07	*0.11	*0.22	<0.07
21/1319	Cow blood	B	No	B-3	2.3	<0.40	*0.08	0.77	1.2	<0.07
21/1322	Cow blood	C	No	C-1	3.5	<0.40	*0.08	1.1	2.1	<0.07
21/1326	Cow blood	C	No	C-2	2.0	<0.40	*0.16	0.48	1.1	<0.07
21/1330	Cow blood	C	No	C-3	3.2	<0.40	<0.07	0.78	2.1	<0.07
21/1334	Cow blood	D	Yes	D-1	2.3	<0.40	<0.07	0.52	1.2	<0.07
21/1338	Cow blood	D	Yes	D-3	2.1	<0.40	<0.07	0.52	1.2	<0.07
21/1342	Cow blood	E	Yes	E-2	2.1	<0.40	0.41	1.5	1.2	<0.07
21/1344	Cow blood	E	Yes	E-3	2.5	<0.40	<0.07	1.2	2.5	<0.07
21/1347	Pig blood	C	No	C-V1	2.6	<0.40	0.37	0.81	4.3	*0.17
21/1349	Pig blood	C	No	C-V3	3.0	<0.40	<0.07	0.57	4.4	0.34
21/1350	Pig blood	C	No	C-V4	1.9	<0.40	<0.08	0.54	3.5	<0.08
21/1351	Pig blood	C	No	C-V5	2.7	<0.40	<0.07	0.55	5.9	<0.07
21/1922[1]	Pig blood	G	No	pig 1	1.7	<0.40	<0.08	1.9	5.5	<0.07
21/1923	Pig blood	G	No	pig 2	2.9	<0.40	<0.07	0.30	3.1	*0.07
21/1924	Pig blood	G	No	pig 3	3.1	<0.40	*0.16	0.79	2.1	*0.09
21/1925[1]	Pig blood	G	No	pig 4	≥8.2[2]	<0.40	<0.07	≥5.6[2]	≥18[2]	<0.07
21/1926	Pig blood	G	No	pig 5	3.1	<0.40	<0.06	1.3	5.7	0.33
21/1927	Pig blood	G	No	pig 6	≥6.7[2]	<0.40	<0.06	2.1	≥7.9[2]	<0.06
21/2762[1]	Pig blood	G	No	pig 7	≥17[2]	<0.40	<0.06	≥10[2]	≥33[2]	<0.06
21/2855[1]	Pig blood	G	No	pig 8	2.3	<0.40	<0.06	3.0	5.0	<0.06

[a] LIMS is VUA sample code

** Value between LOD and LOQ*

[1] Indicative values, because only part of the sample was extracted

[2] Values were higher than the highest calibration standard, and are therefore indicative

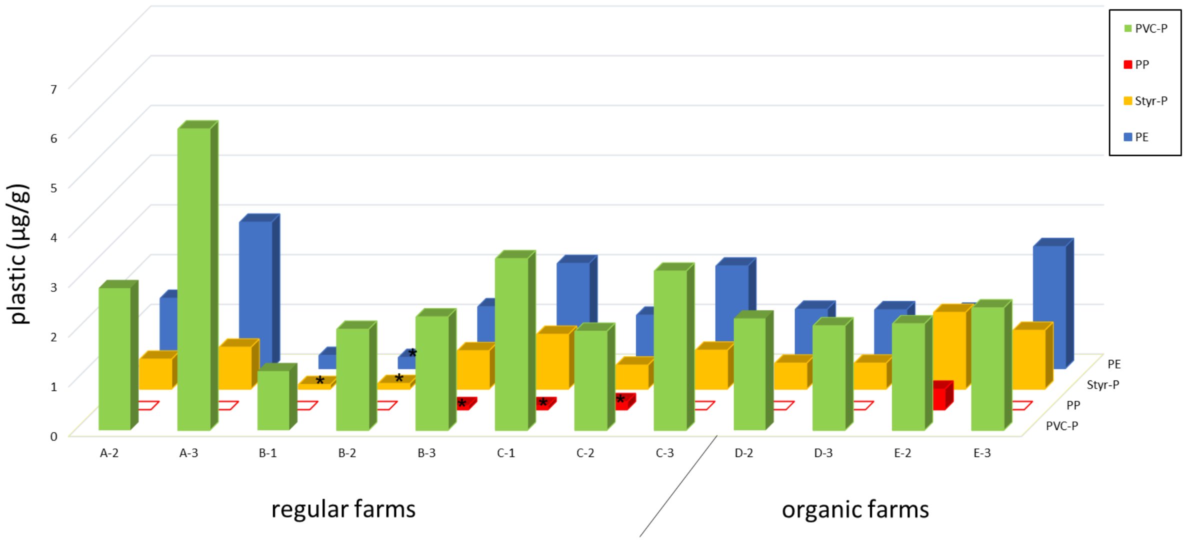

Figure 1 *Plastic concentrations (µg/g) detected in cow blood. PMMA and PET concentrations (all <LOD) are not shown.*
** Value between limit of detection (LOD) and limit of quantification (LOQ)*

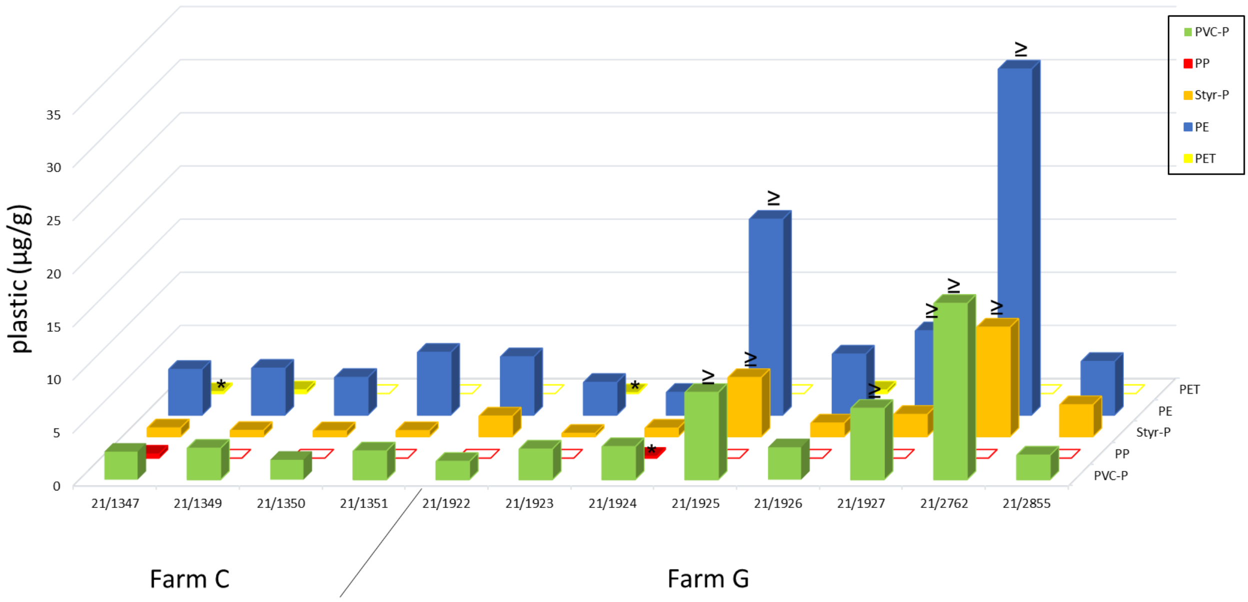

Figure 2 *Plastic concentrations (µg/g) detected in pig blood. PMMA concentrations (all <LOD) are not shown.*
** Value between LOD and LOQ;*
≥ values were higher than the highest calibration point.

The number of blood samples analysed was limited, and therefore the frequency of detection would have had to have been >>1 in 12 samples in order to have a chance of observing any plastic. However, 100% of the cow and pig blood samples contained PVC-P, PE and Styr-P >LOD, so we can say that the number of blood samples was sufficient to show that blood of cows and pigs could contain plastic particles. These results indicate the ‘internal’ exposure of cows and pigs, i.e. the absorbable particles that find their way to the bloodstream. Blood circulates transporting the plastic particles throughout the whole body, where previous laboratory studies have found the potential to be transferred to organs and tissues (Deng et al., 2017; Lu et al., 2018). Based on these findings, it could be concluded that internal exposure of livestock to plastics is common, but no conclusions can be drawn regarding any potential health risks for the animals or for humans upon consumption. This would require further research.

In addition, this study showed a large variation in plastic concentrations between individual animals. A larger number of samples would have to be measured in follow up studies to get a more accurate understanding of the range of plastic concentrations.

3.2 Cow's milk samples

The concentrations of PVC-P, PMMA, PP, Styr-P, PE and PET determined in milk samples obtained from livestock farms, and from supermarkets are given in Table 10, in Appendix C and Figure 3.

PP and Styr-P were not detected in any of the milk samples, PET was only detected in 1 of the 26 samples below LOQ (0.9 µg/g). The highest concentration was detected for PE, which was only detected in two samples (21/2493 full milk from the supermarket, 21/1365 tank milk from farm F). Although those were the highest concentrations (both 21 µg/g) detected in the milk samples, both concentrations were < LOQ and hence considered indicative values due to the high LOD and LOQ for PE.
In addition, for the other two plastics the highest concentrations (PVC-P 13 µg/g; PMMA 0.73 µg/g) were found in the full milk supermarket sample 21/2493. Since only one full milk sample from the supermarket was included in the study, it cannot be concluded that full milk from the supermarket always contains those plastics in the range as detected in this one sample.

All four skimmed milk samples were free of plastics, except PMMA in sample 21/2494 which was detected below LOQ (0.14 µg/g). Of the semi-skimmed milk samples, all detected concentrations were < LOQ except one (PMMA: 0.58 µg/g in sample 21/2484). Only one of the hand milked samples (21/1355) contained one plastic type > LOQ (PMMA 0.52 µg/g). Although none of the tank milk samples contain plastics > LOQ, one of the samples contains a high concentration of PVC-P (12 µg/g) and also some PMMA (0.72 µg/g).

Table 10. Concentrations (µg/g) of 6 polymers analysed in cow's milk, per polymer type.

LIMS code[a]	Sample Type	Farm	Animal Number	PVC-P	PMMA	PP	Styr-P	PE	PET
21/2494	Skimmed milk	Supermarket		<2.1	*0.14	<0.46	<0.02	<14	<0.71
21/2495	Skimmed milk	Supermarket		<2.1	<0.10	<0.46	<0.02	<14	<0.70
21/2497	skimmed, UHT treated	Supermarket		<2.0	<0.10	<0.45	<0.02	<14	<0.70
21/2498	skimmed, UHT treated	Supermarket		<2.1	<0.10	<0.47	<0.02	<15	<0.72
21/2484	semi-skimmed, UHT treated	Supermarket		*6.8	0.58	<0.49	<0.02	<15	<0.74
21/2496	lactose-free semi-skimmed, UHT treated	Supermarket		<1.8	<0.10	<0.41	<0.02	<13	<0.62
21/2485	semi-skimmed milk	Supermarket		<2.2	*0.16	<0.48	<0.02	<15	<0.74
21/2486	semi-skimmed milk	Supermarket		<2.0	*0.21	<0.46	<0.02	<14	<0.70
21/2487	semi-skimmed milk	Supermarket		<2.2	<0.10	<0.49	<0.02	<15	*0.90
21/2488	semi-skimmed milk	Supermarket		<2.0	*0.15	<0.46	<0.02	<14	<0.70
21/2489	semi-skimmed milk	Supermarket		<2.0	<0.10	<0.45	<0.02	<14	<0.69
21/2490	semi-skimmed milk	Supermarket		<2.1	*0.30	<0.47	<0.02	<15	<0.71
21/2491	semi-skimmed milk	Supermarket		<2.1	*0.11	<0.46	<0.02	<14	<0.70
21/2492	semi-skimmed milk	Supermarket		<2.1	*0.16	<0.46	<0.02	<14	<0.70
21/2493	Full milk	Supermarket		13	0.73	<0.46	<0.02	*21	<0.70
21/1355	Milked by hand	Farm A	A-1	*6.1	0.52	<0.44	<0.02	<14	<0.67
21/1357	Milked by hand	Farm B	B-1	<2.0	*0.15	<0.45	<0.02	<14	<0.69
21/1358	Milked by hand	Farm C	C-1	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
21/1359	Milked by hand	Farm D	D-1	<1.9	<0.09	<0.43	<0.02	<13	<0.65
21/1360	Milked by hand	Farm E	E-1	<2.0	*0.15	<0.44	<0.02	<14	<0.68
21/1361	Tank milk	Farm A		<2.0	<0.09	<0.45	<0.02	<14	<0.69
21/1362	Tank milk	Farm B		<2.0	*0.29	<0.46	<0.02	<14	<0.70
21/1363	Tank milk	Farm D		<2.0	*0.11	<0.44	<0.02	<14	<0.68
21/1364	Tank milk	Farm E		<2.0	*0.16	<0.45	<0.02	<14	<0.69
21/1365	Tank milk	Farm F		12	0.72	<0.45	<0.02	*21	<0.69
21/1366	Powder for calves	Farm F		<17	*1.2	<3.9	<0.16	<120	<5.9

[a] LIMS is VUA sample code

** Value between LOD and LOQ*

n.a. not available

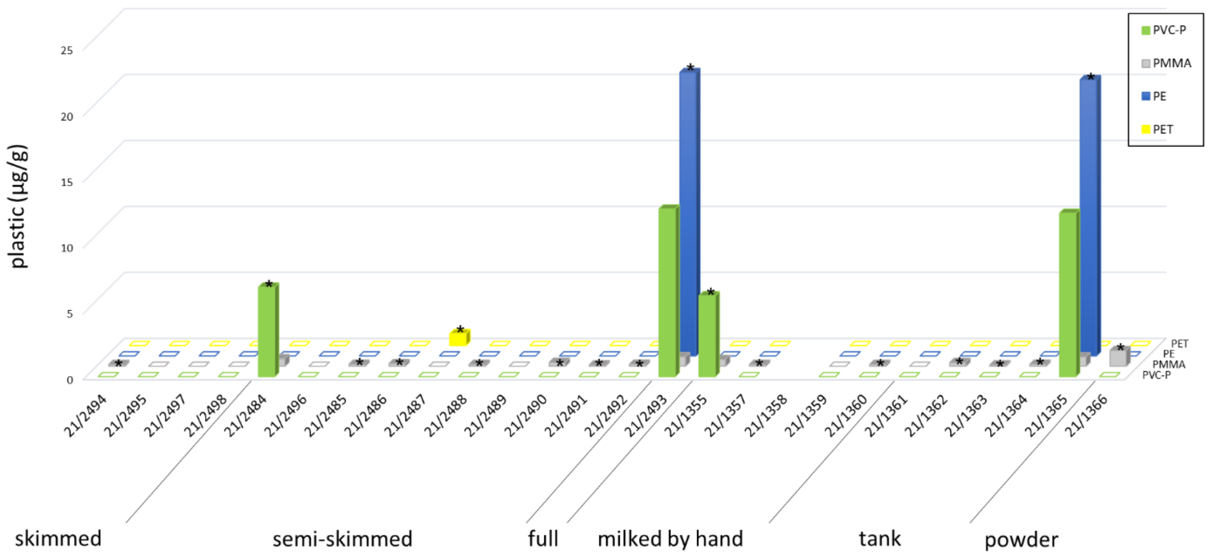

Figure 3 Plastic concentrations detected in cow milk (µg/g). Styr-P and PP concentrations (all <LOD) are not shown.
** = Value between LOD and LOQ.*

In the majority of the milk samples no plastic particles were detected above LOQ. Only four of the 26 tested milk samples contained plastic particles > LOQ. Those four samples were from different origin (supermarket, hand milked and tank milk). Only one full milk sample was analysed, but this sample contained three types of plastic. It is worth investigating more full milk samples to determine whether full milk samples always contain more plastics than other milk samples.

To make a distinction between the milk that may have been contaminated with plastic particles before and after leaving the cow's body, this study included tank milk as well as hand drawn milk and packaged supermarket milk. Considering the low number of samples that contained detectable plastic levels and the different origin of those samples, no general conclusion can be made on the plastic content that was transferred from blood to milk inside the cow's body. Based on these data, no conclusion can be drawn regarding at which point in the pathway - from cow to glass - plastic contamination may have been introduced. Although some of the milk samples do contain one or more plastics types, a general answer cannot be given on the second research question how much plastic is present in Dutch cow's milk.

3.3 Beef and pork samples

Plastic particles were detected in meat samples originating from the supermarket, the butcherie as well as from livestock farms. The concentrations of PVC-P, PMMA, PP, Styr-P, PE and PET determined in the meat samples are given in Table 11, and in Appendix D. The plastic concentrations detected in the meat samples are graphically shown in Figure 4 (Beef) and Figure 5 (Pork). In addition, the moisture content of the meat samples is given in Appendix F.
The majority of the meat samples (7 of 8 beef, and 5 of 8 pork) contained at least one type of plastic. All those samples contained PE. In general, higher concentration of PE were detected in the beef samples (150->7700 µg/g) than in the pork samples (88-690 µg/g). Three of the beef samples (organic burger: 200 µg/g, organic steak: 77 µg/g, fine ribs: 120 µg/g) contained Styr-P, while those polymers were not detected in any of the pork samples. PVC-P was detected in one of the pork samples (Farm C: 130 µg/g), and in 3 beef samples (Farm D: 53 µg/g, organic burger 2600 µg/g, and roasts: 230 µg/g).

In none of the meat samples, PMMA and PET were detected, and PP was only detected in 1 of the pork samples (filet: 63 µg/g).

In this study two organic meat samples were purchased at the supermarket. Both samples contained plastics. One of those organic meat samples contained the highest plastic concentration of all meat samples tested. Since only two organic samples were included in this study, it cannot be concluded based on these results that organic meat samples in general contain more plastic than other meat samples. However, it can be concluded that organic meat can also contain plastic, just as non-organic meat.

Although plastic particles were not present in all beef and pork samples, they were detected in the majority of the meat samples. Hence, it can be concluded that beef and pork do contain plastic particles, which answers research question 3 (Are plastic particles present in beef and pork?).

Table 11. Concentrations (µg/g) of 6 polymers analysed in meat samples, per polymer type.

LIMS code[a]	Sample Type	Farm/ store	Animal	PVC-P	PMMA	PP	Styr-P	PE	PET
21/1387	Beef	Farm B	Cow	<20	<21	<22	<23	330	<24
21/1389	Beef	Farm D	Cow	*53	<18	<19	<20	270	<21
21/2813	round steak	Supermarket	Cow	<19	<20	<21	<21	<20	<23
21/2814	organic burger	Supermarket	Cow	≥2600[2]	<21	<22	200	≥7700[2]	<24
21/2815	organic steak	Supermarket	Cow	<16	<17	<18	77	330	<19
21/2821	round steak	Butchery	Cow	<19	<20	<21	<22	150	<23
21/2819	fine ribs	Butchery	Cow	<15	<16	<17	120	390	<19
21/2820	roasts	Butchery	Cow	230	<19	<20	<20	570	<22
21/1388	pork	Farm C	Pig	130	<22	<23	<24	690	<25
21/2811	ham steak	Supermarket	Pig	<18	<19	<20	<20	110	<22
21/2810	tenderloin	Supermarket	Pig	<17	<18	<19	<19	<18	<21
21/2809	fricandeau	Supermarket	Pig	<18	<19	<20	<20	<19	<22
21/2812	fillet	Supermarket	Pig	<17	<18	63	<19	130	<21
21/2816	ham steak	Butchery	Pig	<17	<18	<19	<19	<18	<21
21/2818	tenderloin	Butchery	Pig	<18	<19	<20	<21	98	<22
21/2817	chop	Butchery	Pig	<17	<18	<19	<19	88	<20

[a] LIMS is VUA sample code

** Value between LOD and LOQ*

[2] Values were higher than the highest calibration point, and therefore indicative

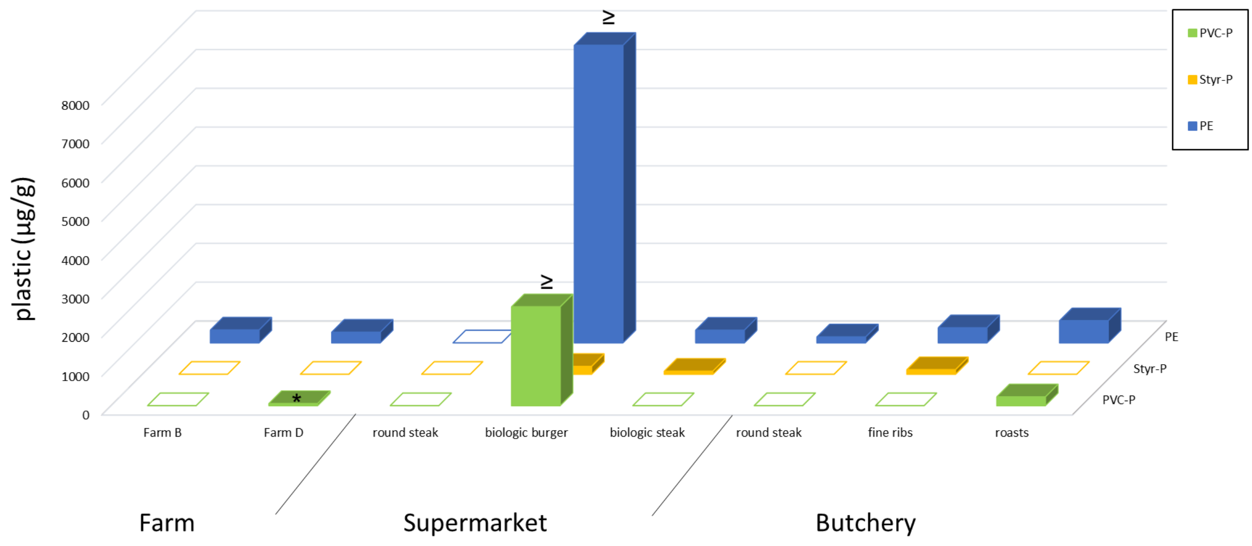

Figure 4 *Plastic concentrations detected in beef (µg/g). PMMA, PP, and PET concentrations (all <LOD) are not shown.*
** Value between LOD and LOQ*
≥ values were higher than the highest calibration point.

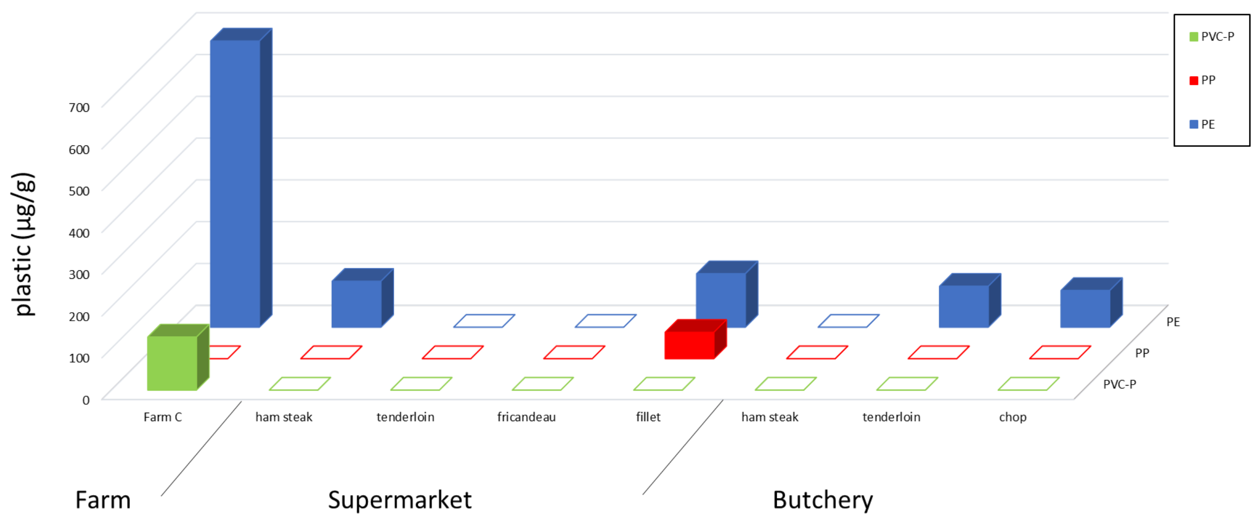

Figure 5 *Plastic concentrations detected in pork (µg/g). Styr-P, PMMA, and PET concentrations (all <LOD) are not shown.*
** Value between LOD and LOQ.*

Since all cow and pig blood samples of part one of this study contained plastic particles, it was expected that also beef and pork would contain plastic particles. Although not for all beef and pork samples, this was the case for the majority of the meat samples (7 of 8 beef, and 5 of 8 pork) in which at least one type of plastic was detected. The plastic types detected in all blood samples were PVC-P, PE and Styr-P. Those were the same types of plastics which were detected in the beef samples. In the pork samples, no Styr-P were detected.

The origin of the plastic in the meats was not determined, but one cannot assume that all of the plastic detected in meat came from the animal's body. This is because all meats had been packaged in plastic (both from farmers and from the supermarket), and the packaging is obviously a potential source of the plastic particles measured in these products, although only the inner part of the purchased piece of meat was taken. Meat processing steps may also be points in the production process in which plastic contamination can be introduced. For plastic particles from meat to be absorbed by humans who ingest meat, the particles must also be small enough to cross biological barriers. Potentially some of the plastic fragments measured in meat were of a size that is invisible to the naked eye yet still not available for absorption and uptake in humans. Since plastic particles were present in meat samples, it is reasonable to assume that humans are exposed to plastic by consumption of meat. However, it should be noted that the preparation of meat e.g. baking, frying, cooking, was not considered in this study. Food processing and preparation may increase or reduce the levels of plastics and subsequent human exposure via the meat.

One of the two organic meat samples analysed contained the highest concentration of plastic particles of all meat samples. Since only two organic meat samples were included in this study, it cannot be concluded that organic meat samples in general contain more plastic than other meat samples. No general conclusion can be drawn about "high" and/or "low" contaminated farms either due to the limited sample size, and the fact that some animals contained higher levels than others.

3.4 Livestock feed samples

The concentrations of PVC-P, PMMA, PP, Styr-P, PE and PET determined in feed samples obtained from livestock farms are given in Table 12, in Appendix E, and in Figure 6.

Plastic particles were not detected in any of the fresh feed samples, while all other feed samples contained at least PVC-P and PE. In addition, Styr-P were detected in all the pellet samples, except one pig pellet sample (21/2795). PMMA, PP and PET were not detected in any of the feed samples tested. There is no observed difference in detected plastic types and concentrations between feed samples taken at regular farms and organic farms.

Table 12. Concentrations (µg/g) of 6 polymers analysed in feed samples, per polymer type.

LIMS code[a]	Sample Type	Farm	Organic (yes/no)	PVC-P	PMMA	PP	Styr-P	PE	PET
21/1375	Cow pellets	A	No	884	<16	<16	220	730	<18
21/1378	Cow pellets	B	No	1000	<15	<16	270	670	<17
21/2794	Cow pellets	C	No	≥2600[2]	<16	<16	740	≥2400[2]	<18
21/1383	Cow pellets	D	Yes	700	<14	<15	52	680	<17
21/1385	Cow pellets	E	Yes	800	<15	<16	191	540	<17
21/1381	Pig pellets	C	No	340	<17	<18	*39	340	<19
21/2795	Pig pellets	G	No	430	<18	<19	<19	400	<20
21/2796	Pig pellets	G	No	700	<16	<17	93	790	<19
21/2797	Pig pellets	G	No	590	<18	<19	*41	960	<20
21/1376	Fresh feed	A	No	<71	<76	<80	<81	<77	<87
21/1380	Fresh feed	B	No	<110	<110	<120	<120	<120	<130
21/1382	Fresh feed	D	Yes	<99	<110	<110	<110	<110	<120
21/2798	Fresh feed	D	Yes	<75	<80	<84	<85	<81	<92
21/1384	Fresh feed	E	Yes	<79	<85	<89	<90	<86	<97
21/2856	Shredded feed	F	No	740	<66	<69	<70	220	<75
21/2857	Shredded feed organic	F	Yes	2000	<71	<74	<75	640	<81

[a] LIMS is VUA sample code

** Value between LOD and LOQ*

n.a. not available

[2] Values were higher than the highest calibration point, and therefore indicative

Figure 6 Plastic concentrations detected in cow and pig feed (µg/g). PMMA, PP, and PET concentrations (all <LOD) are not shown.
** Value between LOD and LOQ*
≥ values were higher than the highest calibration point.

In sample 21/1375 (cow feed pellets, originating from farm A) a macro-plastic was visually detected during sample homogenization (See Figure 7). With FTIR identification, it was determined that the polymer consisted of a mixture of PE and PP. After extraction and analytical analysis with Pyr-GC/MS of the rest of the sample, PP was not detected above LOD. The concentration of PE in this sample was 730 µg/g, which was in the same range as the PE concentration in the other cow pellet samples (540- ≥2400 µg/g).

A) B)

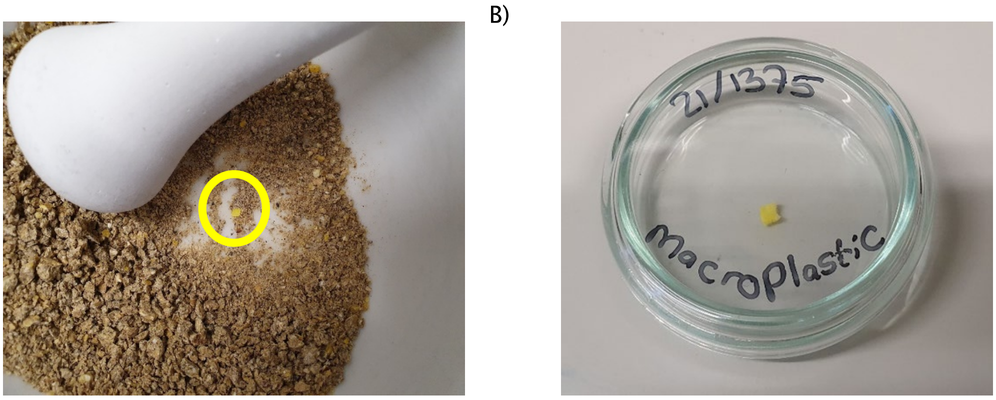

Figure 7 Visually detected plastic macro particle in sample 21/1375 (cow feed pellet from farm A). A: Plastic particle in feed sample during homogenization of the sample; B: plastic particle isolated from sample 21/1375.

The number of feed samples in this pilot study was sufficient to demonstrate that there is a possible exposure of cows and pigs to plastic via their pellet feed. The five fresh feed samples consisting of hay (roughage) did not contain any quantifiable amounts of plastic.

The plastics detected in all feed samples, except fresh feed, were the same types of plastics that were detected most frequently in the beef and pork samples, and in all blood samples. However some are also widely used in packaging and during processing fibers of synthetic textiles and other ambient plastic particles could also contribute to the plastic residues in meat. This suggests that “internal” exposure possibly originates at least partly from the feed.

3.5 Quality assurance and quality control (QA/QC)

3.5.1 Blood samples

Procedural blanks

The six procedural blank samples which were extracted and analysed in the same series as the cow, and pig blood samples contained small amounts of PP (0.02 µg abs) and PE (0.35 µg abs).

The three additional blank samples contained small amounts of PP (0.05 µg abs), PS (0.10 µg abs) and PE (0.47 µg abs).

The blank values were subtracted from the calculated amounts in the samples. Results reported were > 3 x blank values.

Needles and collection tubes

No plastic particles were found in the blank analyses of the needles and the collection tubes.

3.5.2 Cow's milk samples

The five procedural blank samples which were extracted and analysed in the same series as the milk samples contained small amounts of PMMA (0.03 µg abs), PP (0.11 µg abs), PET (0.19 µg abs) and PE (2.30 µg abs).

The blank values were subtracted from the calculated amounts in the samples. Results reported were > 3 x blank values.

3.5.3 Beef and pork samples

No plastic particles were detected in the two procedural blank samples which were extracted and analysed in the same series as the meat samples.

3.5.4 Livestock Feed samples

Only small amount of PET were detected in the blank samples of the pellets (0.63 µg abs.) and the fresh feed samples (0.53 µg abs.) which were extracted and analysed in the same series as the feed samples.

The blank values were subtracted from the calculated amounts in the samples. Results reported were > 3 x blank values.

4 Conclusion

This pilot study gave the first evidence that Dutch pigs and cows are exposed to plastic particles via animal feed pellets, though not via fresh feed. Feed is known as one of the potential uptake routes, along with water consumption and air (respiration). The study did not investigate the origin of the plastic particles detected in the blood streams of pigs and cows, but it did produce evidence that the animals are absorbing plastic particles into their bloodstreams. This indicates that the particles are either small enough to be absorbed via the lung pathway and/or swallowed and absorbed into the blood stream via the gut. Chewing food may further reduce size of particles swallowed which increases the chance of particle absorption (which depends on particles being small enough to pass through biological membranes). The result of all potential exposure routes to plastic particles (i.e. feed, water and air, the latter two of which were not measured in this study) and sources could be found in the bloodstream of all the animals tested. The cows and pigs are exposed to plastic particles that are absorbed into their bodies, and that there is via their feed, although no plastic particles were detected in fresh feed. All Dutch livestock animals that were investigated (12 cows and 12 pigs) had multiple, and at least three types of plastic particles in their blood.

The vast majority of milk samples in this study did not contain detectable, or quantifiable concentrations of plastic particles. Plastic particles were present in the majority (75%) of the beef and pork samples investigated in this study. Therefore, humans may potentially be exposed to plastic particles by eating beef or pork, and less likely via milk.

While this pilot study gives clear indications for plastic exposure of livestock and possibly humans, a larger number of samples would need to be analysed to draw more general conclusions and perform statistical analyses, e.g. regarding the range of concentrations, frequency of detection, temporal and spatial variation in the concentrations. This was beyond the scope of this pilot study.

This pilot study was focused on measuring *exposure* to plastic particles, i.e. the concentrations that could be measured in the samples selected. Based on the results described, no conclusions can be drawn regarding any potential health risks of these findings for the animals or for humans upon consumption of animal-derived products.

5 Acknowledgment

Veterinary doctor Wigert de Pagter of Dierenartsenpraktijk Zwolle Zwartewaterland is gratefully acknowledged for the collection of all farm samples in this study. The anonymous farmers are gratefully acknowledged for their willingness to participate in this study and for the voluntary contribution of various samples from their farms. The VU Animal Ethics Committee is acknowledged for reviewing and approving the project proposal.

6 References

Bureau Risicobeoordeling & Onderzoek (BuRO), 2019. Advies van BuRO over de risico's van de voedergewassen- en diervoederketen. (aan NVWA) Available online: https://www.nvwa.nl/documenten/dier/diervoeder/diervoeder/risicobeoordelingen/advies-van-buro-over-de-risico%E2%80%99s-van-de-voedergewassen--en-diervoederketen#:~:text=binnen%20NVWA%20Zoek-,Advies%20van%20BuRO%20over%20de%20risico's%20van%20de%20voedergewassen%2D%20en,verschillende%20schakels%20van%20de%20diervoederketen.

CBS, 2016. Minder geblaat, meer gemekker op boerderij. https://www.cbs.nl/nl-nl/nieuws/2016/14/minder-geblaat-meer-gemekker-op-boerderij. Access date: 16-03-2022

CBS, 2021a. Rundveestapel per 1 december 2020 (gewijzigd op 30-4-2021). https://www.cbs.nl/nl-nl/cijfers/detail/80274ned. Access date: 16-03-2022

CBS, 2021b. Varkensstapel per 1 december 2020 (Gewijzigd op: 23 april 2021) https://opendata.cbs.nl/statline/#/CBS/nl/dataset/7373LLB/table?fromstatweb. Access date: 16-03-2022

Cortes, C., ESA manual. Mammary gland: physiology and anatomy. Number ZBO101R11510. Available from. https://www.groupe-esa.com/ladmec/bricks_modules/brick01/co/ZBO_Brick01_1.html. Access date: 16/03/2022

Deng, Y., Zhang, Y., Lemos, B., Ren, H., 2017. Tissue accumulation of microplastics in mice and biomarker responses suggest widespread health risks of exposure. *Scientific Reports* 7, 46687. https://doi.org/10.1038/srep46687

Diaz-Basantes, M.F., Conesa, J.A., Fullana, A., 2020. Microplastics in Honey, Beer, Milk and Refreshments in Ecuador as Emerging Contaminants. *Sustainability* 12. https://doi.org/10.3390/su12145514

Dris, R., Gasperi, J., Mirande, C., Mandin, C., Guerrouache, M., Langlois, V., Tassin, B., 2017. A first overview of textile fibers, including microplastics, in indoor and outdoor environments. *Environmental Pollution* 221, 453-458. https://www.sciencedirect.com/science/article/pii/S0269749116312325

FAO, (United Nations Food and Agriculture Organisation) 2021. online publication, Physiology of milk production, Small-Scale Dairy Farming Manual Volume 4 Husbandry Unit 7.1 p. 87 http://www.fao.org/ag/againfo/resources/documents/Dairyman/Dairy/V4U7_1.htm#:~:text=About%20500%20l%20of%20blood,produce%201%20l%20of%20milk. Access date: 2-3-2021

Leslie, H.A., Brandsma, S.H., van Velzen, M.J.M., Vethaak, A.D., 2017. Microplastics en route: Field measurements in the Dutch river delta and Amsterdam canals, wastewater treatment plants, North Sea sediments and biota. *Environment International* 101, 133-142. https://www.sciencedirect.com/science/article/pii/S0160412017301654

Leslie, H.A., van Velzen, M.J.M., Brandsma, S.H., Vethaak, A.D., Garcia-Vallejo, J.J., Lamoree, M.H., 2022. Discovery and quantification of plastic particle pollution in human blood. *Environment International* 163, 107199. https://www.sciencedirect.com/science/article/pii/S0160412022001258

Lu, L., Wan, Z., Luo, T., Fu, Z., Jin, Y., 2018. Polystyrene microplastics induce gut microbiota dysbiosis and hepatic lipid metabolism disorder in mice. *Science of The Total Environment* 631-632, 449-458. https://www.sciencedirect.com/science/article/pii/S0048969718308064

Nederlandse Voedsel- en Warenautoriteit (NVWA), 2018. Aanbieden voerbalen met plastic aan dieren niet toegestaan, Nieuwsbericht | 11-09-2018 | 13:21, https://nvwa.sitearchief.nl/#archive

Appendix A Analysis certificate of plastics in cow blood (µg/g)

Department Environment and Health
De Boelelaan 1108 1081HZ Amsterdam
https://science.vu.nl/en/research/environment-and-health

Certificate of Analysis Microplastics 2021-01 Table 1 of 1

Client	Plastic Soup Foundation
	Sumatrakade 1537
	1019RS Amsterdam
Start analysis	July 2021
Report date	8 October 2021

	EH code	21/1307	21/1310	21/1314	21/1317	21/1319	21/1322	21/1326	21/1330	21/1334	21/1338	21/1342	21/1344
	Code client	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
parameters		µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
PVC-P	Polymers of Polyvinylchloride	2.9	6.1	1.2	2.0	2.3	3.5	2.0	3.2	2.3	2.1	2.1	2.5
PMMA	Polymethylmethacrylate	<0.40	<0.40	<0.40	<0.40	<0.40	<0.40	<0.40	<0.40	<0.40	<0.40	<0.40	<0.40
PP	Polypropylene	<0.08	<0.07	<0.07	<0.07	*0.08	*0.08	*0.16	<0.07	<0.07	<0.07	0.41	<0.07
Styr-P	Polymers of styrene	0.60	0.84	*0.09	*0.11	0.77	1.1	0.48	0.78	0.52	0.52	1.5	1.2
PE	Polyethylene	1.4	2.9	0.26	*0.22	1.2	2.1	1.1	2.1	1.2	1.2	1.2	2.5
PET	Polyethyleneterephthalate	<0.08	<0.07	<0.07	<0.07	<0.07	<0.07	<0.07	<0.07	<0.07	<0.07	<0.07	<0.07

* Value between Limit Of Detection (LOD) and Limit Of Quantification (LOQ)

Date	29 April 2022
Name	Martin van Velzen
Function title	Laboratory Technician

The results are only related to the samples which are made available by the client
This certificate of analysis may only be reproduced in its entirety

Appendix B Analysis certificate of plastics in pig blood (µg/g)

Department Environment and Health
De Boelelaan 1108 1081HZ Amsterdam
https://science.vu.nl/en/research/environment-and-health

Certificate of Analysis Microplastics 2021-01 Table 1 of 1

Client	Plastic Soup Foundation
	Sumatrakade 1537
	1019RS Amsterdam
Start analysis	July 2021
Report date	8 October 2021

	EH code	21/1347	21/1349	21/1350	21/1351	21/1923	21/1924						
	Code client	P1	P2	P3	P4	P5	P6						
parameters		µg/g	µg/g	µg/g	µg/g	µg/g	µg/g						
PVC-P	Polymers of Polyvinylchloride	2.6	3.0	1.9	2.7	2.9	3.1						
PMMA	Polymethylmethacrylate	<0.40	<0.40	<0.40	<0.40	<0.40	<0.40						
PP	Polypropylene	0.37	<0.07	<0.08	<0.07	<0.07	*0.16						
Styr-P	Polymers of styrene	0.81	0.57	0.54	0.55	0.30	0.79						
PE	Polyethylene	4.3	4.4	3.5	5.9	3.1	2.1						
PET	Polyethyleneterephthalate	*0.17	0.34	<0.08	<0.07	*0.07	*0.09						

* Value between Limit Of Detection (LOD) and Limit Of Quantification (LOQ)

Date	29 April 2022
Name	Martin van Velzen
Function title	Laboratory technician

The results are only related to the samples which are made available by the client
This certificate of analysis may only be reproduced in its entirety

Department Environment and Health
De Boelelaan 1108 1081HZ Amsterdam
https://science.vu.nl/en/research/environment-and-health

Certificate of Analysis Microplastics 2021-01 Table 1 of 1

Client	Plastic Soup Foundation
	Sumatrakade 1537
	1019RS Amsterdam
Start analysis	July 2021
Report date	22 December 2021

	EH code	21/1922[1]	21/1925[1]	21/1926	21/1927	21/2762[1]	21/2855[1]						
	Code client	P7	P8	P9	P10	P11	P12						
parameters		µg/g	µg/g	µg/g	µg/g	µg/g	µg/g						
PVC-P	Polymers of Polyvinylchloride	1.7	≥8.2[2]	3.1	≥6.7[2]	≥17[2]	2.3						
PMMA	Polymethylmethacrylate	<0.40	<0.40	<0.40	<0.40	<0.40	<0.40						
PP	Polypropylene	<0.08	<0.07	<0.06	<0.06	<0.06	<0.06						
Styr-P	Polymers of styrene	1.9	≥5.6[2]	1.3	2.1	≥10[2]	3.0						
PE	Polyethylene	5.5	≥18[2]	5.7	≥7.9[2]	≥33[2]	5.0						
PET	Polyethyleneterephthalate	<0.07	<0.07	0.33	<0.06	<0.06	<0.06						

* Value between Limit Of Detection (LOD) and Limit Of Quantification (LOQ)
[1], [2] Values for this sample are indicative values.

Date	29 April 2022
Name	Martin van Velzen
Function title	Laboratory technician

The results are only related to the samples which are made available by the client
This certificate of analysis may only be reproduced in its entirety

Appendix C Analysis certificate of plastics in milk (μg/g)

VRIJE UNIVERSITEIT AMSTERDAM
Faculty of Science
Department Environment and Health
De Boelelaan 1108 1081HZ Amsterdam
https://science.vu.nl/en/research/environment-and-health

Certificate of Analysis Microplastics 2021-02 Table 1 of 2

Client	Plastic Soup Foundation
	Sumatrakade 1537
	1019RS Amsterdam
Start analysis	11 October 2012
Report date	28 October 2021

	EH code	21/2484	21/2485	21/2486	21/2487	21/2488	21/2489	21/2490	21/2491	21/2492	21/2493	21/2494	21/2495	21/2496	21/2497	21/2498
	Code client	Jumbo halfvol	Campina halfvol UHT verhit	Zaanse Hoeve halfvol	Campina halfvol gepasteuri seerd	AH bio halfvol	Demeter zuiver zuivel	Demeter zuiver zuivel	Demeter zuiver zuivel	Demeter zuiver zuivel	Demeter zuiver zuivel	Weerribben Zuivel mager	Zaanse Hoeve mager	Arla lactofree halfvol	AH houdbare mager	Campina langlekker mager
	Tenminste houdbaar tot:	12/10/21	17/01/22	12/10/21	10/10/21	12/10/21	8/10/21	8/10/21	12/10/21	12/10/21	5/10/21	10/10/21	11/10/21	3/03/22	14/03/22	3/03/22
parameters		μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g
PVC-P	Polymers of Polyvinylchloride	*6.8	<2.2	<2.0	<2.2	<2.0	<2.0	<2.1	<2.1	<2.1	13.0	<2.1	<2.1	<1.8	<2.0	<2.1
PMMA	Polymethylmethacrylate	0.58	*0.16	*0.21	<0.10	*0.15	<0.10	*0.30	*0.11	*0.16	0.73	*0.14	<0.10	<0.10	<0.10	<0.10
PP	Polypropylene	<0.49	<0.48	<0.46	<0.49	<0.46	<0.45	<0.47	<0.46	<0.46	<0.46	<0.46	<0.46	<0.41	<0.45	<0.47
Styr-P	Polymers of styrene	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
PE	Polyethylene	<15	<15	<14	<15	<14	<14	<15	<14	<14	*21	<14	<14	<13	<14	<15
PET	Polyethyleneterephthalate	<0.74	<0.74	<0.70	*0.90	<0.70	<0.69	<0.71	<0.70	<0.70	<0.70	<0.71	<0.70	<0.62	<0.70	<0.72

* Value between Limit Of Detection (LOD) and Limit Of Quantification (LOQ)

Date	29 April 2022
Name	Martin van Velzen
Function title	Laboratory technician

The results are only related to the samples which are made available by the client
This certificate of analysis may only be reproduced in its entirety

Department Environment and Health
De Boelelaan 1108 1081HZ Amsterdam
https://science.vu.nl/en/research/environment-and-health

Certificate of Analysis Microplastics 2021-02 Table 2 of 2

Client	Plastic Soup Foundation
	Sumatrakade 1537
	1019RS Amsterdam
Start analysis	11 October 2021
Report date	28 October 2021

	EH code	21/1355	21/1357	21/1358	21/1359	21/1360	21/1361	21/1362	21/1363	21/1364	21/1365	21/1366
	Code client	Hand gemolken melk A	Hand gemolken melk B	Hand gemolken melk C	Hand gemolken melk D	Hand gemolken melk E	Tank melk A	Tank melk B	Tank melk D	Tank melk E	Tank melk F	Melkpoeder kalveren
parameters		µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
PVC-P	Polymers of Polyvinylchloride	*6.1	<2.0		<1.9	<2.0	<2.0	<2.0	<2.0	<2.0	12.0	<17
PMMA	Polymethylmethacrylate	0.52	*0.15		<0.09	*0.15	<0.09	*0.29	*0.11	*0.16	0.7	*1.2
PP	Polypropylene	<0.44	<0.45		<0.43	<0.44	<0.45	<0.46	<0.44	<0.45	<0.45	<3.9
Styr-P	Polymers of styrene	<0.02	<0.02		<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.16
PE	Polyethylene	<14	<14		<13	<14	<14	<14	<14	<14	*21	<120
PET	Polyethyleneterephthalate	<0.67	<0.69		<0.65	<0.68	<0.69	<0.70	<0.68	<0.69	<0.69	<5.9

* Value between Limit Of Detection (LOD) and Limit Of Quantification (LOQ)

Date	29 April 2022
Name	Martin van Velzen
Function title	Laboratory technician
	29 April 2022

The results are only related to the samples which are made available by the client
This certificate of analysis may only be reproduced in its entirety

Appendix D Analysis certificate of plastics in meat (µg/g)

VRIJE UNIVERSITEIT AMSTERDAM
Faculty of Science
Department Environment and Health
De Boelelaan 1108 1081HZ Amsterdam
https://science.vu.nl/en/research/environment-and-health

Certificate of Analysis Microplastics 2022-01 Table 1 of 2

Client	Plastic Soup Foundation
	Sumatrakade 1537
	1019RS Amsterdam
Start analysis	February 2022
Report date	11 March 2022

	EH code	21/1387	21/1388	21/1389	21/2809	21/2810	21/2811	21/2812	21/2813	21/2814	21/2815	21/2816	21/2817
	Code client	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12
parameters		µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
PVC-P	Polymers of Polyvinylchloride	<20	130	*53	<18	<17	<18	<17	<19	≥2600[2]	<16	<17	<17
PMMA	Polymethylmethacrylate	<21	<22	<18	<19	<18	<19	<18	<20	<21	<17	<18	<18
PP	Polypropylene	<22	<23	<19	<20	<19	<20	63	<21	<22	<18	<19	<19
Styr-P	Polymers of styrene	<23	<24	<20	<20	<19	<20	<19	<21	200	77	<19	<19
PE	Polyethylene	330	690	270	<19	<18	110	130	<20	≥7700[2]	330	<18	88
PET	Polyethyleneterephthalate	<24	<25	<21	<22	<21	<22	<21	<23	<24	<19	<21	<20

* Value between Limit Of Detection (LOD) and Limit Of Quantification (LOQ)

[1], [2] Values for this sample are indicative values.

Date	29 April 2022
Name	Martin van Velzen
Function title	Laboratory technician

The results are only related to the samples which are made available by the client
This certificate of analysis may only be reproduced in its entirety

Department Environment and Health
De Boelelaan 1108 1081HZ Amsterdam
https://science.vu.nl/en/research/environment-and-health

Certificate of Analysis Microplastics 2022-01 Table 2 of 2

Client Plastic Soup Foundation
Sumatrakade 1537
1019RS Amsterdam

Start analysis February 2022

Report date 11 March 2022

	EH code	21/2818	21/2819	21/2820	21/2821								
	Code client	M13	M14	M15	M16								
parameters		µg/g	µg/g	µg/g	µg/g								
PVC-P	Polymers of Polyvinylchloride	<18	<15	230	<19								
PMMA	Polymethylmethacrylate	<19	<16	<19	<20								
PP	Polypropylene	<20	<17	<20	<21								
Styr-P	Polymers of styrene	<21	120	<20	<22								
PE	Polyethylene	98	390	570	150								
PET	Polyethyleneterephthalate	<22	<19	<22	<23								

* Value between Limit Of Detection (LOD) and Limit Of Quantification (LOQ)
(1), (2) Values for this sample are indicative values.

Date 29 April 2022
Name Martin van Velzen
Function title Laboratory technician

The results are only related to the samples which are made available by the client
This certificate of analysis may only be reproduced in its entirety

Appendix E Analysis certificate of plastics in feed (µg/g)

VRIJE UNIVERSITEIT AMSTERDAM
Faculty of Science
Department Environment and Health
De Boelelaan 1108 1081HZ Amsterdam
https://science.vu.nl/en/research/environment-and-health

Certificate of Analysis Microplastics 2022-01 Table 1 of 2

Client Plastic Soup Foundation
Sumatrakade 1537
1019RS Amsterdam

Start analysis December 2021

Report date 7 February 2022

	EH code	21/1375	21/1378	21/2794	21/1383	21/1385	21/1381	21/2795	21/2796	21/2797			
	Code client	Cow pellets 1	Cow pellets 2	Cow pellets 3	Cow pellets 4	Cow pellets 5	Pig pellets 1	Pig pellets 2	Pig pellets 3	Pig pellets 4			
parameters		µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g			
PVC-P	Polymers of Polyvinylchloride	880	1000	≥2600[(2)]	700	800	340	430	700	590			
PMMA	Polymethylmethacrylate	<16	<15	<16	<14	<15	<17	<18	<16	<18			
PP	Polypropylene	<16	<16	<16	<15	<16	<18	<19	<17	<19			
Styr-P	Polymers of styrene	216	270	740	52	190	*39	<19	93	*41			
PE	Polyethylene	730	670	≥2400[(2)]	680	540	340	400	790	960			
PET	Polyethyleneterephthalate	<18	<17	<18	<17	<17	<19	<20	<19	<20			

* Value between Limit Of Detection (LOD) and Limit Of Quantification (LOQ)
(1), (2) Values for this sample are indicative values.

Date 29 April 2022
Name Martin van Velzen
Function title Laboratory technician

The results are only related to the samples which are made available by the client
This certificate of analysis may only be reproduced in its entirety

Department Environment and Health
De Boelelaan 1108 1081HZ Amsterdam
https://science.vu.nl/en/research/environment-and-health

Certificate of Analysis Microplastics 2022-01 Table 2 of 2

Client	Plastic Soup Foundation
	Sumatrakade 1537
	1019RS Amsterdam
Start analysis	December 2021
Report date	7 February 2022

	EH code	21/1376	21/1380	21/1382	21/2798	21/1384	21/2856	21/2857					
	Code client	Fresh food-1	Fresh food-2	Fresh food-3	Fresh food-4	Fresh food-5	Shredded food-1	Shredded food-2					
parameters		µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g					
PVC-P	Polymers of Polyvinylchloride	<71	<100	<99	<75	<79	740	2000					
PMMA	Polymethylmethacrylate	<76	<110	<110	<80	<85	<66	<71					
PP	Polypropylene	<80	<120	<110	<84	<89	<69	<74					
Styr-P	Polymers of styrene	<81	<120	<110	<85	<90	<70	<75					
PE	Polyethylene	<77	<120	<110	<81	<86	220	640					
PET	Polyethyleneterephthalate	<87	<130	<120	<92	<97	<75	<81					

* Value between Limit Of Detection (LOD) and Limit Of Quantification (LOQ)

Date	29 April 2022
Name	Martin van Velzen
Function title	Laboratory technician

The results are only related to the samples which are made available by the client
This certificate of analysis may only be reproduced in its entirety

Appendix F Moisture content of meat samples (% of wet weight)

LIMS code[a]	Sample Type	Farm/ store	Animal	Moisture (%)
21/1387	beef	Farm B	Cow	71
21/1389	beef	Farm D	Cow	75
21/2813	round steak	Supermarket	Cow	73
21/2814	organic burger	Supermarket	Cow	64
21/2815	organic steak	Supermarket	Cow	75
21/2821	round steak	Butchery	Cow	73
21/2819	fine ribs	Butchery	Cow	75
21/2820	roasts	Butchery	Cow	74
21/1388	pork	Farm C	Pig	71
21/2811	ham steak	Supermarket	Pig	74
21/2810	tenderloin	Supermarket	Pig	74
21/2809	fricandeau	Supermarket	Pig	74
21/2812	fillet	Supermarket	Pig	73
21/2816	ham steak	Butchery	Pig	77
21/2818	tenderloin	Butchery	Pig	75
21/2817	chop	Butchery	Pig	75

[a] LIMS is VUA sample code