SLUTRAPPORT

CirculIns

Circular handling of plastic waste by the Insects .

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Förord

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The project was run within Norbite with main participants Nathalie Berezina and Maxim Chapovalov. Importantly, several stakeholders have been also involved in the discussions about the implementation of the project, the interpretation of results, and their overall dissemination. We can thus cite: Paul Bussmann from Wageningen University, Frans van den Berg from Oost NL, Toon Ansems from Cirkelwaarde, Walter Jansen and Willem Jan from Amusca, Graham Aid and Robert Norgren from Ragn Sells, Krister Essvik from RISE, and Svetlana Eskebaek from Packbridge.

Innehåll

1. Sammanfattning

Projektet CirculIns tar upp den påträngande frågan om den linjära plastindustrin där mindre än 10% av plast återvinns, vilket leder till miljöskador. Det brådskande behovet av övergång till en cirkulär och resurseffektiv plastindustri kan inte överdrivas. CirculIns tar upp denna utmaning med ett enskilt fokus på oåtervinningsbar plastavfall och erbjuder innovativa lösningar som lovar att omvandla denna miljöbelastning till en värdefull resurs.

I kärnan av CirculIns-metoden ligger användningen av *Galleria mellonella*-larver, en specifik insektart, för att omvandla plast till tre avgörande komponenter: proteiner, lipider och biogödsel. Denna omvandlingsprocess har resulterat i en rad anmärkningsvärda prestationer och djupa påverkningar.

Noterbara milstolpar har uppnåtts:

1. Förbättrad omvandling av larver med en potentiellt patenterbar prediktiv modell.
2. Upptäckt av nya produkter under förädlingen av larver.
3. Efterlevnad av europeiska standarder för husdjursfoder för larvinnehåll.
4. Säker larvdieter från olika avfallsströmmar som avslöjar värdefulla spårämnen.
5. Strategisk planering av immateriella rättigheter genom konkurrensanalys.
6. Engagemang med intressenter, vilket resulterar i samarbetsmöjligheter.

Dessa prestationer stärker kopplingarna mellan plastproducenter, avfallshantering och användare av färdiga produkter. Kortsiktiga resultat inkluderar partnerskap med företag som Infinity Fibers (FI) och pågående samarbete med RISE (SE). Den långsiktiga visionen innefattar en automatiserad industriell demonstrationsanläggning som kan bearbeta 5 000 ton omöjligt återvinningsbart plastavfall per år, vilket möjliggör både industriell expansion och en licensieringsmodell.

Trots dessa anmärkningsvärda framgångar har CirculIns stött på utmaningar som förtjänar ytterligare undersökning och innovation. Bland dessa finns problemet med larvdödlighet, vilket kräver noggrann hantering genom förbättrad vatten/fuktighetstillförsel och finjustering av larvtäthet vid olika utvecklingsstadier.

Dessutom finns det en skyldighet att genomföra en omfattande massbalansanalys inom systemet, vilket innebär en kvantitativ utvärdering av insektavfall. Ytterligare tester och analys krävs för det nyligen separerade produkten som härstammar från larvbiorefining, och särskilda ansträngningar krävs för att förbättra tekniker för insektslakt.

När vi blickar mot framtiden är CircuIIns inställd på att påbörja kritiska steg mot den industriella implementeringen av Norbites banbrytande teknologi. Under de kommande månaderna syftar projektet till att skala upp processen och lägga grunden för utvecklingen av en industriell demonstrationsanläggning inom en tvåårig tidsram. Slutligen sätter CircuIIns sikte på det monumentala målet att kommersialisera teknologin inom fem år.

Sammanfattningsvis utgör CircuIIns en banbrytande kraft i strävan efter hållbar hantering av plastavfall. Den smider viktiga kopplingar, främjar innovation och förespråkar en cirkulär och resurseffektiv plastindustri. Genom detta erbjuder den inte bara en vision om en mer hållbar framtid utan också en konkret väg framåt för att nå dit.

Larver av *Galleria mellonella* som konsumerar polyuretanavfall

2. Summary

The CircuIIns project addresses the pressing issue of the linear plastic industry, where less than 10% of plastics are recycled, leading to environmental harm. The urgent need for a transition to a circular and resource-efficient plastic industry cannot be overstated. CircuIIns takes up this challenge with a singular focus on unrecyclable plastic waste, offering innovative solutions that hold the promise of transforming this environmental scourge into a valuable resource.

Central to the CircuIIns approach is the utilization of *Galleria mellonella* larvae, a specific insect species, to effect the conversion of plastics into three critical components: proteins, lipids, and bio-fertilizers. This transformative process has yielded an array of remarkable achievements and profound impacts.

Notable milestones have been achieved within Circulins:

1. Enhanced larval transformation with a potentially patentable predictive model.

2. Discovery of a novel product during larval refinement.

3. Compliance with European pet food standards for larval content.

4. Safe larval diet from various waste streams, revealing valuable trace elements.

5. Strategic intellectual property planning through competitive analysis.

6. Engagement with stakeholders, resulting in collaboration opportunities.

These achievements strengthen connections among plastic producers, waste management entities, and end-product users. Short-term outcomes include partnerships with companies like Infinity Fibers (FI) and ongoing collaboration with RISE (SE). The long-term vision involves an automated industrial demonstrator processing 5,000 tons/year of non-recyclable plastic waste, enabling both industrial expansion and a licensing model.

Notwithstanding these notable successes, CirculIns has encountered challenges that merit further investigation and innovation. Among these is the issue of larval mortality, which demands precise management through improved water/humidity supply and the fine-tuning of larval density at various developmental stages.

Additionally, there is an imperative to conduct a comprehensive mass balance analysis within the system, entailing a quantitative evaluation of insect waste, known as frass. Further testing and analysis are required for the newly separated product derived from larval biorefining, and dedicated efforts are necessary to refine techniques for insect slaughtering.

Looking to the future, CirculIns is set to embark on critical steps toward the industrial implementation of Norbite's transformative technology. In the coming months, the project aims to scale up the process, laying the groundwork for the development of an industrial demonstrator within a two-year timeframe. Ultimately, CirculIns sets its sights on the momentous goal of commercialization within a five-year horizon.

In sum, CirculIns stands as a pioneering force in the quest for sustainable plastic waste management. It forges vital connections, fosters innovation, and champions the cause of a circular and resource-efficient plastic industry. In doing so, it offers not only a vision for a more sustainable future but also a tangible roadmap to get there.

Larvae of *Galleria mellonella* consuming polyurethane waste

3. Inledning och bakgrund

Over the past decades, global population and income growth has led to a significant increase in material consumption. On a current global scale, almost 400 million tonnes of plastic turn into waste annually, including packaging, textile, and furniture, at postconsumer and industrial levels. This number is anticipated to increase, through the combination of plastic production- and plastic waste growth, corresponding to 3,4% and 3,65 CAGR , respectively. In order to meet the UN's 2050 goal of climate neutrality, there is a clear and pressing need to reduce the environmental impact of the plastic industry.

The plastic industry operates in an almost completely linear way: large amounts of nonrenewable resources are extracted to produce packaging, textile, and furniture, some of those products are used for only a short time, after which the materials are mostly sent to landfill or incinerated. Important to mention that even the plastic materials produced from renewable resources are not necessary biodegradable, e.g. bio-PE, NIPU, etc., which means that the handling of their end-of-life remains an issue in the same way as their fossil-based counterparts.

There is a need to improve plastic sorting capabilities and to better manage plastic waste. Innovation is required around the methods to deal with plastics at different stages. In terms of recycling technologies, the fundamental question is the molecular composition of plastics and polymeric materials. Progress on recycling methods into new plastics has mostly been done by developing and applying mechanical or chemical recycling processes on pure monomeric plastic waste, e.g. PET plastic bottles. Having in mind that the global plastic production when it comes to molecular type has the following distribution : 16,7% PP, 15,7% LDPE, 14,5% PP&A fibres, 12,3% HDPE, 9,3% PVC, 8,1% PET, 6,6% PUR, 6,1% PS, the main recycling difficulties concern the smooth plastic materials, such as LDPE, fibres, such as polyester/polyamide/polyacrylate and thermosets (non-melting plastics), such as PUR, for most of these materials there is no valuable chemical or physical recycling process available to date. The different plastic types are often blended in the same material (i.e. polycotton, elastane content etc) adding on to the challenges with complex material streams and issues/difficulties in separating the blended components, efficiency of separation, quality of separated material and hence viability of recycling. To summarize, there will be a huge need of different types of recycling and valorising processes to be able to channel the many different plastic waste fractions to a suitable process. The polymer-to-polymer recycling processes will set high standards on incoming plastic waste streams, and there will still be large quantities of plastic waste streams that will be very difficult to recycle into new plastics, i.e. beddings and pillows, fossil-based fabrics containing many different types of fibres, polyurethane-containing multi-layer textiles etc.

Galleria mellonella, also known as great wax moth, was recently shown to possess a unique and outstanding capacity of consuming and transforming used polymeric materials such as polyethylene or polystyrene . Utilising this moth's capacity opens up potentialities in the plastic industry to manage the complexity of plastic recycling, especially as it offers the possibility of handling unsorted end-of-life plastic streams.

This specific capacity of the insects comes from the remarkable molecular similitude between the natural substrate of the moth – beeswax, and the molecular structure of commonly used plastics. Norbite has been extensively working on the understanding of the scope of the degradation capacity of G. mellonella and have demonstrated that this insect can digest more than 80% of commonly used polymeric materials, including recalcitrant synthetic fibres such as polyamides, polyesters or polyacrylates, as well as their mixes and co-polymers.

Moreover, at the end of the transformation the high-quality proteins and lipids to be used for food and feed applications are obtained, and even the frass, insects' poop, can be used as biofertilizer, making this process a perfect example of circular economy.

Yet, insects are considered as farm animals by the EU, therefore the substrates that can be fed to them are regulated. The main challenge is the mainstreaming of resource efficiency, as current policy does not support non-agricultural leftovers and end-of-life products entering the value chain again. Development of new circular business models requires regulatory change. To move forward, Circulins will deepen knowledge in this area of feeding substrate - products' properties relationship, i.e. specific characteristics of the products obtained by breeding the insects on plastic waste streams. Circulins will thus serve as a platform for taking next step in industrialisation and commercialisation of circular economy pathways.

Circulins is an applied research project exploring a novel and disruptive approach towards end-of-life plastics and polymeric materials, specifically residual plastics for which there is no viable re-use or recycling solutions, based on the capacity of the insect Galleria mellonella to digest and transform plastics into proteins. Instead of disposal, the conventional end-of-life scenario in a linear plastic value chain, the project has developed the technological foundations to allow residual plastics to be utilised as a food source for the insect *Galleria mellonella*. As the larvae of the insect can be refined for multiple high-value products, including proteins and fibres; lipids; fertilisers; antimicrobial peptides, the utilisation of plastic waste in this way gives it the opportunity to re-enter the economy. In short, the insect's transformation capabilities mean the elimination of residual products in the plastic industry through their conversion into input material for another value-creating one i.e. industry symbiosis.

The project is based on the principles of resource efficient development, specifically the conversion of waste into a resource. As a product is produced, value is added as more materials, energy, and labour are utilised in the production process. Once the product reaches its end-of-use however, the value created during the production process is almost completely lost due to current waste management practices. Resources that could otherwise be recycled and reused are simply disposed of or incinerated. Circulins's overall vision is a plastics' economy in which packaging, textiles, and furniture are kept at their highest value during use and re-enter the economy afterwards, never ending up as waste. This vision complements ongoing efforts to make the plastic industry more sustainable by minimising its negative impacts. With specific emphasis on innovation towards a different system, our project presents an opportunity to deliver substantially better economic, societal, and environmental impact in the short, and long, term in comparison to current waste management practices.

Circulins extends beyond technological development. We are aware that transforming the industry requires system-level change with collaboration and a commitment to innovation from multiple actors, together with public authorities and civil society. The operationalisation of the project's systemic perspective can be seen most significantly through the inclusion of a reference group comprising actors along the plastic value chain and end users of the larvae transformation process. Project results were disseminated to reference groups members in a way which promotes shared learning between actors with differing expertise and encourages multi-stakeholder alliances.

Issues of particular interest are the needs, requirements and actions needed to sustain the new value chain which this project's innovation is bringing about. For example, plastics free of hazardous chemicals are a prerequisite for the transformation process, thus simultaneous actions are needed to eliminate hazardous chemicals from the manufacturing supply chain in the long-term.

4. Genomförande

Circulins was conducted within NBtech AB, coordinated by Nathalie Berezina and with participation of Maxim Chapovalov. The project duration was 12 months, and the main work was divided into three work packages, that run as follows:

- WP1: transformation of larvae of *G. mellonella*. This WP concerns the thermo-mechanical treatment of the larvae at their end of growth period, with subsequent separation and characterization of the main ingredients, i.e. proteins and lipids. It is important to mention that the complete characterization of the obtained products is mandatory, as chemical composition gives indications on the composition of the product and its nutritional value, i.e. suitability of the products for feed applications; the biological characterization validates the sanitation aspects of the developed transformation process, whereas the physical characterization contributes to assess the suitability of the obtained products for further processing steps required by our customers, feed formulators, for the production of final products. According to the obtained results, the process was continuously improved, and several iterations have been made during the course of the project

- WP2: IPR studies. This WP concerns the way to deal with the results of the project to increase the impact of the overall process. First aspect is to assess the IPR and deal with the confidentiality in order to improve the competitiveness of Norbite on the global plastic waste treatment and protein production market.

- WP3: Communication. This WP concerns another important aspect, i.e. communication about the results and their dissemination to a broader audience, once the required IPR studies are fulfilled. To achieve this objective, we have built a reference group composed from upstream and downstream users of the project's users, i.e. plastic and polymer producers, waste treating companies, and petfood and aquaculture formulators – users of the obtained protein- and lipid-based products. Regular meetings, every 6 months during the project duration with the representative from the reference group will allow us to inform them about our achievements and also to learn from them about suitability of our products and process and contribute to the improvement of the suitability of our solution to their specific needs. The reference group will also play a multiplying role in the dissemination of the results and increasing the awareness around the project and its results, thus broading the audience towards specific stakeholders and other actors within the concerned industries.

5. Resultat och diskussion

5.1. Transformation of insects, WP1

For the transformation of larvae of *G. mellonella*, WP1, two main aspects have been studied: thermo-mechanical transformation of larvae, and the analysis of their intricinsic composistion.

5.1.1. Thermo-mechanical processing of larvae

The thermo-mechanical processing of laravae transformation consist fo several steaps, namely, slaughtering of larvae, their mincing, i.e. homogeneisation, and separation of the main metabolites, i.e. solid and liquid proteins, and lipids. Among those steps two have been subjected to a more scrutinized study: slaughtering and separation.

5.1.1.1. Slaugthering of larvae

Slaughtering is an important step as it concerns the actual killing of larvae, and therefore needs to be performed with the highest ethical standards in the matter. Yet, there is no established rules/procedures for the slaugthering of artropds, as some insect producers prvilige direct mincing of the animals, while others are passing through a boiling step, yet boiling of some artropodes, e.g. lobsters, have been already forbidden in some jurisdictions, e.g. Switzerland.

IPIFF, an interprofessional organization within EU working to promote the utilization of insects as food and feed, has published some guidelines on the processing of the insects, yet they remain rather evasive when talking about slaughtering.

Two different techniques for slaughtering of the insects have been studied during CirculIns, and one was found to be more efficient than the other, as thus methods remain confidential for more detailed description see section **Error! Reference source not found.**. Yet even the newly chosen solution is not completely satisfactory, therefore further invistigations of these aspects are to be foreseen.

5.1.1.2. Separation of larval content

Separation of proteins and lipids is another important step in the processing of insects' larvae. Usually, it is achieved through several steps, water addition, cooking, and either a tricantation or a double decantaion to separate the three main components, lipids, aquesous phase containing soluble proteins, and a solid phase. Further optional steps may consit in oil polishing, aqueous phase concentration, its mixing and drying together with solid proteins.

During the CirculIns project we have focused our attention on separation itself, i.e. optimisation of water addition, cooking and tricantation steps. Our approach aimed to simplify this process by applying a "all in one" procedure. The developed method represents an important know-how for Norbite, therefore it is detailed in section **Error! Reference source not found.**. An important outcome that we may underline, is that besides an important simplification of the processing step, our method also allowed the separation of a foruth product, which is currently under characterization for future commercialisation as an additional asset.

5.1.2. Analysis of larval composition

The analysis of larval composition is crucial for the fine-tuning of our solution ot the sepcfici market needs in terms of applications of proteins in lipids. Therefore, different aspects have been studied, larval composition from the nutrional point of view, composition of raw materials on which larvae have been fed, and the influence of aw materials on larvae compoeition.

5.1.2.1. Analysis of whole larvae

The general analysis of whole larvae showed nice equilibrium between lipids and proteins, quasi-absence of ash and fibers, and moisuture of almost 60%. The quasi-absence of antrinutritional elements, allowed pepesic deigestility to be as high as 90,4% (Table 1).

Table 1. General characterisation of larval composition

characteristic	value
moisture	59.9%
ash	0.9%
crude fiber	1.4%
crude fat	23%
crude protein	16.6%
pepsic digestibility	90.4%

A more detailed look into fatty acids composing *G. mellonella*'s fat shows that the predominant fattay acids are oleic, palmitic, and linoleic, with 40,2%, 29,4% and 19,2%, respectively (Figure 1). While a-linolenic, stearic and palmitoleic acids are found in minor proportions. This gives a total of unsaturated fatty acids, UFAs, at 67,2% of total lipids present in *G. mellonella*, and a total of polyunsaturated fatty acids, PUFAs at 23%, with a global ratio of Omega 6 to Omega 3 at 8,9.

As a somparison, olive oil presents 82,3% of UFAs and only 13,3% of PUFAs, thus showing the high quality of oil obtained after refining of larvae of *G. mellonella*.

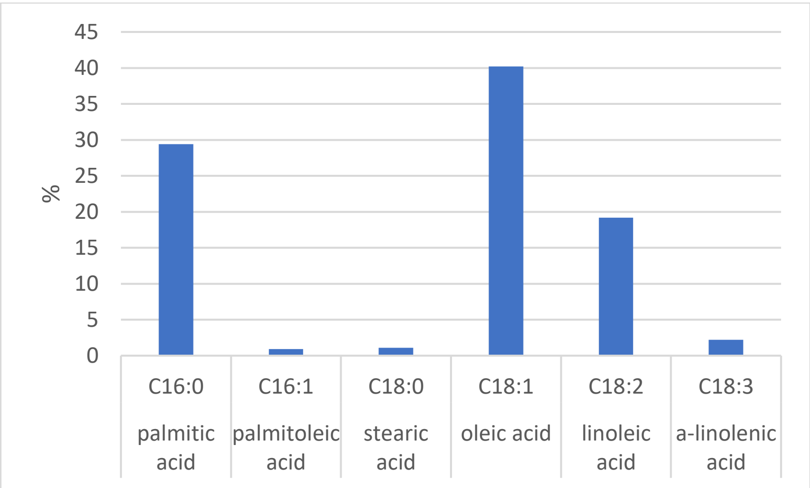

Figure 1. Fatty acids distribution within *G. mellonella*'s fat

A more detailed analysis of *G. mellonella*'s protein shows a well equilibrated distribution of different aminoacids (Figure 2).

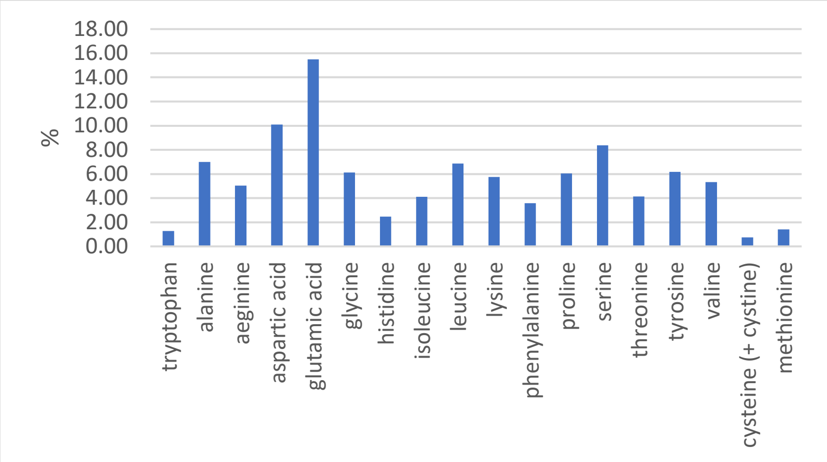

Figure 2. Aminoacids distribution within *G. mellonella*'s protein

A thorough comparison of the amonoacid distribution within the proteins of *G. mellonella* with the recommendations of the FEDIAF – the European Pet Food Industry (Table 2) shows that apart for the sulfur-continaing aminoacids, i.e. methionine and cysteine, where some supplementation is suitable, the overall composition of *G. mellonella*'s protein is perfect for all types of dog food. Concerning the cat food, apart for arginine, where a supplementation is required for all types of cat food, *G. mellonella*'s protein is perfect for a highly caloric adult cats' food. For low caloric adult cats' food as well as for growth and reproduction cats' food additional supplementations in leucine, tyrosine, and sulfur-containing aminoacids is suitable.

Table 2. Comparison of the amonoacid distribution within the proteins of *G. mellonella* with the recommendations of the FEDIAF – the European Pet Food Industry

Aminoacid *(g/100 g DM)*	**Dog food**				**Cat food**			***G. mellonella***
	Early growth (<14 weeks)	Late growth	Adults 110 kcal ME/kg	Adults 95 kcal ME/kg	Growth & Repro	Adults 100 kcal ME/kg	Adults 75 kcal ME/kg	
Arginine	0,82	0,74	0,52	0,60	1,07	1,00	1,30	0,734
Histidine	0,39	0,25	0,23	0,27	0,33	0,26	0,35	0,359
Isoleucine	0,65	0,50	0,46	0,53	0,54	0,43	0,57	0,6
Leucine	1,29	0,80	0,82	0,95	1,28	1,02	1,36	1
Lysine	0,88	0,70	0,42	0,46	0,85	0,34	0,45	0,838
Methionine	0,35	0,26	0,40	0,46	0,44	0,17	0,23	0,206
Methionine+cysteine	0,7	0,53	0,76	0,88	0,88	0,34	0,45	0,315
Phenylalanine	0,65	0,50	0,54	0,63	0,50	0,40	0,53	0,523
Phenylalanine+tyrosine	1,30	1,00	0,89	1,03	1,91	1,53	2,04	1,423
Threonine	0,81	0,64	0,52	0,60	0,65	0,52	0,69	0,603
Tryptophan	0,23	0,21	0,17	0,20	0,16	0,13	0,17	0,187
Valine	0,68	0,56	0,59	0,68	0,64	0,51	0,68	0,778

Green: *G. mellonella*'s protein provides at least enough of the considered amonoacid
Black: *G. mellonella*'s protein provides almost enough of the considered amonoacid
Red: *G. mellonella*'s protein does not provide enough of the considered amonoacid

5.1.2.2. Analysis of raw materials

The raw materials from the two tested waste streams, i.e. sludge of soft packaging and polyurethane, PUR, have been thouroughly studied. Those streams were specifically tested for two sets of potential pollutnats, i.e. heavy metals and flame retardant agents.

When analysing metallic composition of mixture, it is important to keep in mind that not all the metals hav ethe same impact on human and animal health. Thus, three categories of metals are usually distinguished: heavy metals, minerals, and trace elements.

Heavy metals are usually considered as dangerous at certain doses and are to be avoided. There are five have metals that are usually considered in food and feed assessment: mercury, lead, arsenic, chromium, and cadmium.

Minerals, on the contrary, are metals that are important for human and animal development, there presence is usually required for a well-equilibrated diet. The metals entering this category are calcium, potassium, sodium, and magnesium. Although important for the nutritional assessment of prodicuts, these metals have not been subject of the current study.

Finally, trace elements, are metals which repsence even in small amounts is required for a healthy development of organisms. The trace elements usually considered in food and feed applications are copper, iron, manganese, selenium, and zinc.

A thorough analysis of heavy metals and trace elements have been realised on the used waste streams, i.e. sludge containing smooths packaging, and two different wastes composed of polyurethane, PUR 1 and PUR 2; as well as on wax as reference (Table 3).

Table 3. Metal composition of different waste streams and wax, used as reference

metal	unit	PUR 1	PUR 2	Sludge	wax
Heavy metals					
As	mg/kg	<0.1	<1.27	<0.1	<1.15
Cd	mg/kg	<0.02	<0.25	0.03	<0.23
Cr	mg/kg	<0.1	0.87	1.86	<0.58
Hg	mg/kg	<0.05	<0.51	<0.05	<0.46
Pb	mg/kg	<0.2	1.16	0.486	<0.23
Trace elements					
Ba	mg/kg	nd	2.13	nd	<1.00
Co	mg/kg	0.0298	2.37	0.0849	0.25
Cu	mg/kg	0.55	2.86	34.6	<0.30
Mn	mg/kg	0.398	<5.0	4.26	<5.0
Ni	mg/kg	<0.2	<1.3	0.971	<1.2
Zn	mg/kg	8.42	11.4	142	<2.3

PUR 1 & PUR 2 correspond to two different PUR-containing waste streams
Sludge corresponds to a waste treating company sludge mainly containing residues of smooth packaging

The performed analyses have shown several aspects. An important difference has been observed between the two polyurethane-containing waste streams, e.g. presence of recorded amounts of chromium and lead in PUR 2, thereas none of them has been detected in PUR 1. Also, among heavy metals arsenic and mercury were absent in all the samples, and only traces of cadmium have been reported in sludge, while chromium and lead present main polluting heavy metals present in recorded amounts in PUR 2 and sludge (Table 3).

At the same time the presence of different trace elements has been reported in all the samples, traces of cobalt, for example, being even reported in wax. Among the different trace elements, the presence of zinc has been reported at a most significant amounts, reaching up to 142 mg/kg in the sludge (Table 3).

A set of 13 flame-retardant agents has been also analysed (Table 4). Among all those molecules, only tris(chloropropyl)phosphate, TCPP, has been detected, and only in the PUR 1 waste streams. The amount of the detected TCPP remains low, 1,4 mg/kg, yet it is important to follow-up on this and other flame-retardant molecules as an accumulation effect can occur while insects are feeding on the selected waste streams.

Table 4. Flame retardant composition of different waste streams and wax, used as reference.

Flame retardant agent	**unit**	**PUR 1**	PUR 2	**RS**	**wax**
tris(klorpropyl)fosfat (TCPP)	mg/kg	1.4	<1.0	<1.0	<1.0
tris(2-kloroetyl)fosfat (TCEP)	mg/kg	<1.0	<1.0	<1.0	<1.0
tris(1,3-diklor-2-propyl)fosfat (TDCP)	mg/kg	<1.0	<1.0	<1.0	<1.0
tributylfosfat (TBP)	mg/kg	<1.0	<2.0	<1.0	<1.0
tris(2-butoxietyl)fosfat (TBEP)	mg/kg	<1.0	nd	<1.0	nd
tris(2-etylhexyl)fosfat (TEHP)	mg/kg	<1.0	nd	<1.0	nd
tri-isobutylfosfat (TiBP)	mg/kg	<1.0	nd	<1.0	nd
trikresylfosfat (TCrP)	mg/kg	<5.0	<5.0	<5.0	<5.0
tri-o-kresylfosfat (ToCrP)	mg/kg	<1.0	nd	<1.0	nd
trifenylfosfat (TPHP)	mg/kg	<1.0	<1.0	<1.0	<1.0
dibutylfenylfosfat (DBPhP)	mg/kg	<2.0	nd	<2.0	nd
difenylbutylfosfat (DPhBP)	mg/kg	<1.0	nd	<1.0	nd
2-etylhexyldifenylfosfat (EHDPhP)	mg/kg	<1.0	nd	<1.0	nd

PUR 1 & PUR 2 correspond to two different PUR-containing waste streams
Sludge corresponds to a waste treating company sludge mainly containing residues of smooth packaging

5.1.2.3. Analysis of influence of raw materials on larval composition

Larvae at instar 5-6 have been submitted for 2 weeks to a feeding based on waste streams and on wax as a reference, their composition in terms of metal and flame-retardant content has been then analysed (Table 5, Table 7)

Cocnerning the metal composition, no presence of heavy metal has been detected in any of the examined batches; while a presenc of several trace elements, i.e., copper, manganese, and zinc, has been observed in all batches, including the one performed on wax.

These observations are rather interesting, first they show that there is no presence of heavy metals in larvae after feeding for a two-week period on different waste streams, even containing non neglictible amount of some of these metals, e.g. chromium and lead (Table 3). This opens a way for a more continuous study of potential influence of heavy metals on a longer term, with larvae at earlier stages of development.

Interestingly, the presence of trace elements has been observed in all batchesm including the one fed with wax (Table 5). The amounts of the observed trace elements are also rather consistent, with only exception for copper. These observations request a more thorough study to better understand what is the source of those trace elements within the larvae and is there a maximum amount, for certain of them at least, that larvae can accumulate.

Table 5. Metal composition of larvae fed with different waste streams and wax, used as reference

metal	unit	larvae on PUR 1	larvae on PUR 2	larvae 1 on sludge	larvae 2 on sludge	larvae on wax
Heavy metals						
As	mg/kg	<0.08	<0.08	<0.08	<0.1	<0.08
Cd	mg/kg	<0.005	<0.005	<0.005	<0.007	<0.005
Cr	mg/kg	<0.03	<0.03	<0.03	<0.04	<0.03
Hg	mg/kg	<0.01	<0.01	<0.01	<0.01	<0.01
Pb	mg/kg	<0.04	<0.04	<0.04	<0.06	<0.04
Trace elements						
Ba	mg/kg	nd	nd	nd	nd	nd
Co	mg/kg	<0.005	<0.005	<0.005	<0.007	<0.005
Cu	mg/kg	5.8	5.67	6.28	8.14	4.95
Mn	mg/kg	1.17	0.839	0.85	1.17	1.63
Ni	mg/kg	<0.04	<0.04	<0.04	<0.06	<0.04
Zn	mg/kg	32.7	30.3	31.8	38.3	22.1

PUR 1 & PUR 2 correspond to two different PUR-containing waste streams
Larvae 1 on sludge and larvae 2 on sludge correspond to replicates with different larval batches on a waste treating company sludge mainly containing residues of smooth packaging

Another important aspect that needs to be assessed after the consideration of those results, concerns the overall mass balance of heavy metals, as they have not been observed within the larvae, they might be in the frass, the poe, of the animals.

These results on trace elements have to be also put in relation with the recommendations of the FEDIAF – the European Pet Food Industry (Table 6). These results show that trace elements found in larvae of *G. mellonella* cover the needs of all categories of cats and dogs considered by the FEDIAF. Importantly, the trespassing of higher limits for some of the diets is not problematic as larvae will not be utilised as such bit within the formulation, i.e. the trace elements content will be diluted to reach the corresponding limits.

Table 6. Recommendation of the FEDIAF – the European Pet Food Industry for the trace element content of pet food

Trace element *(mg/100 g DM)*	**Dog food**				**Cat food**		
	Early growth (<14 weeks)	Late growth	Adults 110 kcal ME/kg	Adults 95 kcal ME/kg	Growth & Repro	Adults 100 kcal ME/kg	Adults 75 kcal ME/kg
Copper	1,10	>1,10 <2,80	>0,72 <2,80	>0,83 <2,80	>1,00 <2,80	>0,50 <2,80	>0,67 <2,80
Iron	8,80	>8,80 <68,18	>3,60 <68,18	>4,17 <68,18	>8,00 <68,18	>8,00 <68,18	>10,70 <68,18
Manganese	0,56	>0,56 <17,00	>0,58 <17,00	>0,67 <17,00	>1,00 <17,00	>0,50 <17,00	>0,67 <17,00
Selenium	40,00	>40,00 <56,80	>18,00 <56,80	>22,00 <56,80	>30,00 <56,80	>21,00 <56,80	>28,00 <56,80
Zinc	10,00	>10,00 <22,70	>7,20 <22,70	>8,34 <22,70	>7,50 <22,70	>7,50 <22,70	>10,00 <22,70

A thorough analysis of flame retardant agents within the larvae fed for 2 weeks with different waste streams showed that no flame-retardant agent was present in their final composition (Table 7).

As for the heavy metals, these results open a way for a more continuous study of potential influence of flame retardant agents on a longer term, with larvae at earlier stages of development.

Table 7. Metal composition of larvae fed with different waste streams and wax, used as reference

Flame retardant agent	unit	larvae on PUR 1	larvae on PUR 2	larvae 1 on sludge	larvae 2 on sludge	larvae on wax
tris(klorpropyl)fosfat (TCPP)	mg/kg	nd	nd	nd	nd	nd
tris(2-kloroetyl)fosfat (TCEP)	mg/kg	nd	nd	nd	nd	nd
tris(1,3-diklor-2-propyl)fosfat (TDCP)	mg/kg	nd	nd	nd	nd	nd
tributylfosfat (TBP)	mg/kg	nd	nd	nd	nd	nd
tris(2-butoxietyl)fosfat (TBEP)	mg/kg	nd	nd	nd	nd	nd
tris(2-etylhexyl)fosfat (TEHP)	mg/kg	nd	nd	nd	nd	nd
tri-isobutylfosfat (TiBP)	mg/kg	nd	nd	nd	nd	nd
trikresylfosfat (TCrP)	mg/kg	nd	nd	nd	nd	nd
tri-o-kresylfosfat (ToCrP)	mg/kg	nd	nd	nd	nd	nd
trifenylfosfat (TPHP)	mg/kg	nd	nd	nd	nd	nd
dibutylfenylfosfat (DBPhP)	mg/kg	nd	nd	nd	nd	nd
difenylbutylfosfat (DPhBP)	mg/kg	nd	nd	nd	nd	nd
2-etylhexyldifenylfosfat (EHDPhP)	mg/kg	nd	nd	nd	nd	nd

PUR 1 & PUR 2 correspond to two different PUR-containing waste streams
Larvae 1 on sludge and larvae 2 on sludge correspond to replicates with different larval batches on a waste treating company sludge mainly containing residues of smooth packaging

5.2. IPR studies, WP2

An entomoindustry overview was directed towards the technical field of breeding and farming of insects.

The object of the entomoindustry overview was to identify key actors in Norbite's competitive landscape in the field of breeding and farming of insects. A further object of this overview was to recommend which key actor(s) constitute interesting candidates for a competitor's watch/monitoring for Norbite.

Hence, this report provided relevant data relating to patent portfolios of key actors already identified in a previous landscaping search provided by Norbite, assessed for publications from 2018 and onwards. Relevant data relating to possible new actors was further provided in this report based on citations and new classifications resulting from the above-mentioned assessment of already identified key actors.

From the above, several key actors could be derived as interesting candidates for a potential watch/monitoring for Norbite. For the Asian key actors and for the new key actors, IP portfolios held by universities are very local in terms of IP rights. With the assumption that universities generally do not intend to build substantive businesses with their IP portfolios, Norbite focuses its monitoring towards industrial key actors. That being said, universities should not be disregarded as their IP portfolios are relevant in terms of the prior art it creates which could be cited against future Norbite patent applications.

The key actors to monitor have been designed as Ynsect-Protifarm and Protix-Buhler, and their portfolios thoroughly analysed.

This survey also governed the IP development strategy of Norbite, with the identification of both key actors and key regions where the insect industry is developing in the fastest way. A compromise also needs to be found between the IPR protection and the financial means to ensure this protection, also considering the possibilities of potential future legal actions in case of infringement of IPR.

The implementation of the IPR strategy has been translated into entering of national phases of the EP20187902 and EP20187892 patents of Norbite and the future entering in the national phase, currently in PCT, of Norbite's patents EP21183517.8 and EP21183520.2 (Table 8).

Table 8. Current status of Norbite's IP portfolio

Id	**title**	**EP/WO number**	**filing date**	**PCT**	National phase				
					Europe	**USA**	**China**	**Singapore**	**Tunisia**
NBT-P1	Apparatus for growing biomass	EP20187902	2020	2022	2023	2023	2023	2023	2023
NBT-P2	System and method for growing biomass	EP20187892	2020	2022	2023	2023	2023		
NBT-P3	Biomass production	EP21183517.8	2021	2023	tbd	tbd	tbd	tbd	tbd
NBT-P4	Plastic digestion	EP21183520.2	2021	2023	tbd	tbd	tbd	tbd	tbd

5.3. Communication, WP3

The focus was made on broadcasting the non-confidential results and outcomes generated during the project to both wide and relevant audience with the aim to heighten awareness about the achievements, to contribute to the promotion of knowledge-based, resource efficient and more inclusive industry, and to ensure that as many relevant stakeholders as possible have access to these results.Activities regarding dissemination & communication of the project results were primarily oriented towards (1) enhancing the eventual commercial potential of the final product and business model, (2) notifying the scientific and industry-specific R&D sectors of the project's results (3) informing actors at different points in the plastic value chain, as well as end users of the results, of the value of the project processes and outputs.

The project continuously engaged discussion with relevant stakeholders during the project period to make sure that the project work and results can be utilized and exploited, and to get input and advice from a broad perspective.

In total we have participated in 21 external communication events, of which 12 oral communications, 4 invited lectures, 2 poster presentations, and 4 stakeholder meetings (Table 9). Moreover, 24% of these events were held abroad, while 43% were in Sweden, and the rest on-line.

Table 9. Summary of participation to external communication events

Type of participation	Name of the event	Place	Date
Oral communication	Insects in food and feed	Borlänge, Sweden	August 2022
Oral communication	Livsmedel in focus	Tylosend, Sweden	September 2022
Oral communication	Agri Food'ture challenge	Dijon, France	September 2022
Oral communication	Scanpack	Gothenbourg, Sweden	October 2022
Oral communication	at Barkarby Science morning	Barkarby, Sweden	October 2022
Invited lecture	Harper Adams University	Birmingham, UK	November 2022
Poster presentation	Slush	Helsinki, Finland	November 2022
Oral communication	Stuns	Uppsala, Sweden	November 2022
Invited lecture	ENSCMu	on-line	November & December 2022
Oral communication	The New 2030 Food Paradigm	Paris, France	December 2022
Invited lecture	Aarhus University	on-line	December 2022
Invited lecture	AgroParisTech	on-line	January 2022
Stakeholder meeting	Go West	Gothenbourg, Sweden	February 2023
Oral communication	Packbridge	Malmö, Sweden	March 2023
Stakeholder meeting	I3 project	Apelboorn, Netherlands	April 2023
Oral communication	Insects for Food and Feed	on-line	April 2023
Poster presentation	Nordic Sustainability Expo	Stockholm, Sweden	May 2023
Oral communication & Stakeholder meeting	Plastindustriforening meeting	Malmö, Sweden	May 2023
Oral communication	RE:source dagen	on-line	May 2023
Stakeholder meeting	I3 project	on-line	May 2023
Oral communication	Future protein production	on-line	May 2023

6. Slutsatser, nyttiggörande och nästa steg

6.1. Summary of results

The CirculIns project has exceeded expectations with remarkable achievements, including:

1. Enhancing the thermo-mechanical process of larval transformation and developing a predictive mathematical model for system efficiency. These findings are currently under scrutiny for potential patenting.

2. Discovering a novel, promising product during larval refinement.

3. Thoroughly characterizing larval content and confirming its compliance with European pet food industry standards.

4. Demonstrating that a 2-week larval diet consisting of various waste streams, including smooth packaging and polyurethane, does not introduce heavy metals or flame-retardant agents into the larval content. Instead, promising trace elements like zinc, copper, and manganese have been identified.

5. Conducting a comprehensive competitive analysis and refining our intellectual property rights (IPR) strategy accordingly.

6. Actively participating in numerous national and international events and engaging in four dedicated stakeholder meetings to disseminate our research results and facilitate future scientific, technological, and commercial collaborations.

6.2. Circulins's impact

The primary impact of these findings lies in enhancing the connectivity among various stakeholders within the targeted value chain, encompassing plastic producers, those grappling with plastic waste challenges (such as waste treatment companies and municipalities), and end-product users, particularly feed and food formulators offered by Norbite.

In the short term, this has already resulted in increased collaboration, exemplified by a submitted proposal with Infinity Fibers, a Finnish company specializing in recycling used cotton textiles, and another proposal in development with RISE, focusing on the upcycling of old shoes.

In the long term, this heightened interconnectivity is poised to culminate in the establishment of a fully automated industrial demonstrator capable of converting up to 5,000 tons per year of non-recyclable plastic waste into proteins, lipids, and biofertilizers. This strategic development will enable the implementation of a dual go-to-market approach, encompassing both actual industrial expansion and a licensing model, facilitating the rapid proliferation of our technology.

6.3. Future prospects

Several challenges have arisen during the project, necessitating further investigation. These include addressing larval mortality by optimizing water/humidity levels and adjusting larval density at various developmental stages.

Additionally, there is a need for a comprehensive mass balance analysis within the system, involving quantitative assessment of insect waste (frass. Further, the newly separated product from larval biorefining requires additional analysis and testing, along with focused efforts on insect slaughtering techniques.

Looking ahead, the critical steps toward the industrial implementation of Norbite's technology involve scaling up the process in the coming months, progressing to the development of an industrial demonstrator within a two-year timeframe, and ultimately achieving commercialization within five years.

7. Publikationslista

Two articles have been published so far:

1, "Plastavfall blir till foder och växtnäring – med hjälp av en unik larv" in Grönt Sammhällsbyggande, June 2023, https://www.grontsamhallsbyggande.se/2023/06/14/plastavfall-blir-till-foder-och-vaxtnaring-med-hjalp-av-en-unik-larv/

Summary: Get rid of plastic waste that cannot be recycled - in a way that simultaneously generates food, plant nutrition and other useful products? It works, with the help of a very special caterpillar. In the RE:Source-funded project Circulins, the company Norbite wants to take the next step with its unique business idea.

2, "De äter knepiga plaster" in Avfall och Miljö, September 2023, https://ebooks.exakta.se/avfall_sverige/2023/2304/#zoom=true

Summary: The wax moth may save plastic recycling and at the same time produce food in a sustainable way. Behind this discovery stands Norbite, a Swedish start-up company that now aims to scale-up its process.

8. Projektkommunikation

The dissemination of the project results was done by all the participants of the project. This includes attending (inter)national events open to external participants and the publishing of content. The communication strategy (see WP2) addressed the division of dissemination activities, providing a breakdown of opportunities to disseminate results and communicate on the project, as well as a timeline to be followed. The extent to which results and activities are spread was coordinated with the IPR studies and following the advice of the IP subcontractor (AWA patent).

The reference group acted as a multiplier for project results. Norbite communicated results to the reference group during stakeholder meetings over the course of the project. The reference group members contextualised the information received so as to communicate it in a tailored way relevant to their unique audiences, thereby contributing to more nuanced dissemination.

A visual identity was developed that includes a logo, font and colours that were compiled in a style guide. Based on this visual identity, templates were created for project outputs such as reports and presentations. This enabled cohesive dissemination and communication of the project.

In total we have participated in 21 external communication events, of which 12 oral communications, 4 invited lectures, 2 poster presentations, and 4 stakeholder meetings. Moreover, 24% of these events were held abroad, while 43% were in Sweden, and the rest on-line. For more detailed list of actions and reached audiences see Table 9 and paragraph 5.3.

9. Referenser

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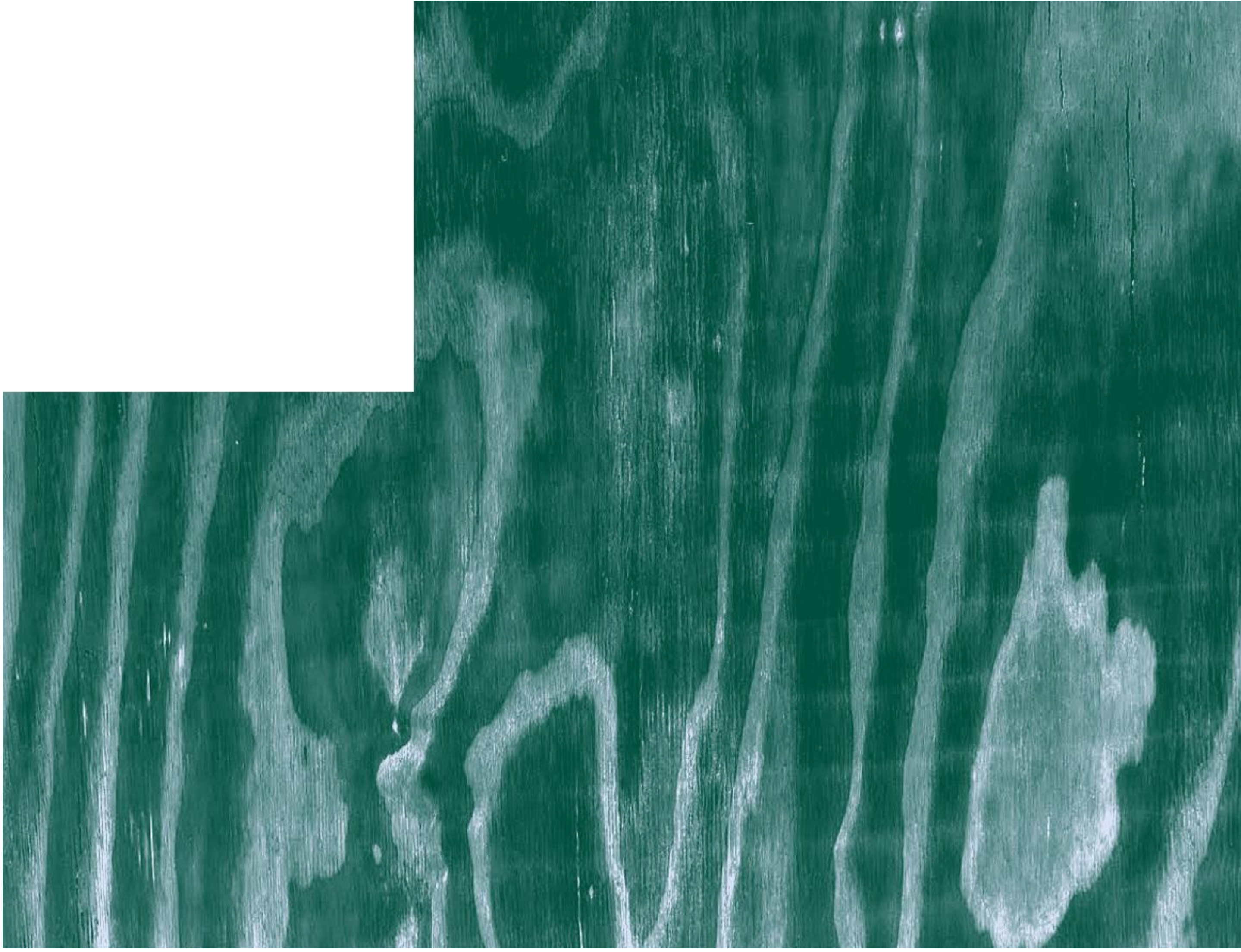

RE:Source är ett strategiskt innovationsprogram som fokuserar på att utveckla cirkulära, resurseffektiva materialflöden. Vårt mål är att uppnå en hållbar materialanvändning där vi håller oss inom planetens gränser.