Episode 73: Pesticides - Flowers, Food and Forensics

Transcript

Hi everyone, welcome back to the Tox Lab. I'm Rebecca. I'm Rob. This week we're going to be taking a look at pesticides, which is, I'm going to be honest, not an area I am mega familiar with.

What about you Rebecca? Yeah, I'm definitely not super familiar with pesticides either. Well, I thought we could explore that a little bit before we dive into our papers. I've got a little quiz for you.

I'm going to ask you some questions about pesticides. Oh, we do enjoy a quiz. Because I must admit, I actually went down some serious rabbit holes prepping for this episode. That doesn't surprise me.

DDT wasn't originally a pesticide. True or false? True. True.

So it was actually discovered many, many years before it was realized it was a pesticide. And DDT, if you're not familiar with DDT, is a very infamous pesticide used to control mosquito populations and it turned out it started affecting lots of downstream animals through bioaccumulation. Nicotine is a pesticide. I want to say false. So this is true. Nicotine, a naturally occurring compound in tobacco plants, acts as a pesticide and stops insects eating the plant.

Rotenone is used for fishing. You don't know what rotenone is. No, it rings a bell, but I want to say false. So this is actually true.

So rotenone is a compound which is actually extracted from roots. It actually has a history of being used as a fishing aid because it's a selective fish poison. That's not the right word. Piscicide, I think is the right word.

Okay. And you throw it in a river and fish float to the surface because it poisons the fish. Okay. I feel like I'm not doing very well on this quiz.

You're not doing very well at all. Okay. Finally, one more. Historic pesticide.

Now we used to use lead arsenate on apples. Oh, I'm going to guess and say true. True. So remarkably, a lead arsenic salt, so double poison, was used widely in orchards and we'd spray the apples and then we'd eat the apples.

We are going back a few years. It seems like a bad idea. So before we dive properly into this week's papers, I do want to give a big shout out to Sandrine who contacted us and recommended this episode off the back of an article in The Guardian looking at pesticide exposure in florists. We also have a little announcement.

If you want to, you can now support us through Buy Me A Coffee, so you can find us on buymeacoffee.com forward slash The Tox Lab. Huge shout out to everyone who's donated so far. We really, really appreciate it. Absolutely no pressure to support us in that way.

Listening and sharing is plenty. Yeah, we've no plans to paywall primary content or anything like that. But you know, if you want to support us, we do really appreciate that too. And thank you, yeah, to those who have already done so.

It really does mean a lot to us. So why don't we dive in then and look at our first paper this week? So flower production uses a wide range of pesticides to achieve production targets and research published in 1979 showed that 17.7% of all flowers imported to Miami contain pesticide residue levels greater than five parts per million and that three lots had levels greater than 400 parts per million. A study in 2014 in Belgium showed that cut flowers are heavily contaminated with 107 active substances being detected and a total average pesticide load of 15.73 milligrams per kilogram of flowers.

And there seems to be less restriction on the use of pesticides in flowers compared with foods, and there is a lack of regulation and maximum residue limits. Cut flowers are generally sprayed at a maximum dosage up to the time they are harvested and then shipped directly to markets with no delay between treatment and harvesting. So this really was the background to this Guardian article, wasn't it? And I must admit, it took me by surprise.

I don't have a huge amount to do with pesticides. Occasionally, we encounter them in our clinical work and our forensic work, but in general, that's not something we tend to do a lot of. But what I am aware of is there are a lot of colleagues out there doing pesticide residue testing in drinking water and in food. It had never occurred to me that flower production may actually be a significant source of exposure.

So there are potential acute and chronic effects of pesticides after exposure during spraying or post harvest. And the UN environmental program estimates that globally 20,000 workers die each year from pesticide poisoning after direct or indirect exposure. Prostate and testicular cancers in male fluoroculture pesticide applicators have been reported and cervical cancer has also been reported in females in Florida. 60% of long-term workers in the fluorocultural industry in Italy have had early stage cancers and genetic damage has been reported in more than 71% of cut flower growers worldwide.

I mean, that's massive! I know, it was the stat that really shocked me when I read it. The amount of pesticide residue on the hands of workers represents the main measure of dermal exposure. Many pesticides that are sprayed onto flowers are in the form of persistent fat-soluble pesticide residues, which can be dislodged from the surface of the plant after spraying, as the active substances are absorbed and fixed on the surface of the plant.

In this paper that we're going to discuss, they aim to measure the transfer of pesticide residues from flowers to hands and to assess the potential dermal exposure to florists, and they looked at the risk level of each active substance by comparing those to the acceptable operated exposure levels. I feel like we need to clarify a little bit before we go too far into this one. When we're talking about pesticides, what are we talking about? We're talking about a mixture here, aren't we, of insecticides, so compounds that control plant-eating bugs and insects, antifungals, herbicides.

Yeah, they're a whole class. So we're not talking about one thing, we're talking about hundreds, possibly more? Thousands, maybe? Possibly.

Of compounds, all of which are used to varying degrees in various different countries. So in this study, cotton gloves were distributed to 20 volunteer florists in Belgium who wore these for two consecutive half days when handling flowers and preparing BKs for a minimum of two hours but a maximum of three hours a day, and this was to evaluate the potential dermal exposure from pesticide transfer from flowers to hands.

The gloves were then weighed, cut into small pieces and transported to a lab who used both GCMS and LCMS for analysis, and they then calculated the potential dermal exposure values for the substances and then converted that into systemic doses. So all glove samples were contaminated by high levels of pesticide residues for most active substances and a total of 111 were identified with an

average of 37 per sample and an average total pesticide residue concentration per glove sample of 22.22 mg per kilogram. The concentrations of pesticides present were that of a thousand times higher than what is normally detected in food.

So really significant concentrations of pesticides and not just one compound but lots of compounds. Half of the active substances detected were fungicides, the other half insecticides, and there was one growth regulator and one herbicide detected. 14 active substances were the most frequently detected and were present on more than 75% of the samples, and these names are really long and slightly hard to pronounce, so apologies if I butch these. But these included azoxystrobene, venomol, boscolid, clofentazine, phenhexamide, flonicamide, fludate, ioxanil, fluopiram, imidacloprid, opridione, ufenarone, methiocarb, prosimidone, and spiroxamine.

So I'm going to be completely honest. The only one of those I think I've heard of is methiocarb. And I think that's because it's in our high res library, and if I'm searching for methamphetamine, I will type meth first. So when looking at potential dermal exposures at the maximum concentrations, five compounds exceeded the acceptable operator exposure levels, and at maximum values for systemic exposure, four compounds exceeded the acceptable operator exposure level, and these were clofentazine, fomoxidone, methiocarb, and pyridovin.

And the maximum systemic exposure for clofentazine was four times higher than the acceptable operator exposure level. So these are dose exposures that are mandated in maximum exposures for people working with these compounds? Yes, I believe so. So people who are, for example, agricultural workers, shouldn't be exposed to doses beyond this, and yet florists were handling flowers that were in doses beyond that.

But when looking at actual dermal exposure when florists are using PPE such as gloves, the levels did not exceed the acceptable operator exposure levels that are outlined. So it seems that by using PPE, you can dramatically reduce the risk of pesticide exposure. And this makes sense, doesn't it? Yeah.

Because in a lab, we're so used to wearing gloves all the time. We're handling all sorts of things, but we've always got our gloves on. But thinking about it, you're right, you know, you go to a high street florist and people make you up a bouquet of flowers. Gloves aren't what you imagine, and I suspect a lot of the time, people aren't wearing gloves when handling these flowers.

And certainly if I buy a bunch of flowers and bring it home, I don't think I need to put my gloves on before I put this bunch of flowers in a vase. Yeah, exactly. Okay, so let's drill into this a little bit then. Florists are exposed, particularly if they're not wearing gloves, and some of these exposures could be very significant.

What is the risk to florists? I think it's quite tricky to pin down a lot of the pesticides that were breaching those maximum systemic exposure limits were at quite high concentrations that you're not really seeing typically in cut flowers. They were like the extremes in the West Coast scenario. So it is a little bit challenging to work out exactly what that risk is.

But then there is potentially, if you're being exposed to multiple different pesticides that work in a similar way, are you then putting yourself at an increased risk of harm? I think that's the real challenge with these, isn't it? We're not talking about acute toxicity. We're not talking about somebody touching a flower and feeling unwell.

We are talking about chronic systemic accumulated toxicity. Very lipophilic compounds potentially accumulating within the person as well. Coming into work, handling flowers day in, day out. It's the chronic exposure that could potentially lead to chronic long-term effects, including some of these increased cancer risks.

And you were saying at the start, weren't you, that there are some documented epidemiological cases whereby people who work with cut flowers or people who work in horticulture are at increased risk of cancer. So there is real potential here for significant increased risk to long-term health. But as you've said, gloves potentially might resolve a lot of that. So why don't we now look at a slightly different angle and take a look at food?

Because food, unlike flowers, is a lot more tightly regulated. So this paper is looking at pesticide residues in bananas and kiwi fruits from Turkey. So both bananas and kiwi fruits are susceptible to disease pressures. So pesticide-based control strategies are implemented to keep up with the yield and quality of the fruit.

And there's concern over accumulation of pesticide residues in these fruits and the risk associated to consumers. So this study looked at a broad spectrum of 235 pesticide residues, which included insecticides, fungicides, herbicides, acaraicides, and plant growth regulators and assessed the occurrence and levels of pesticide residues in bananas and kiwis in Turkey along with their compliance with EU maximum residue levels. They also looked at deterministic and probabilistic estimations of chronic dietary exposure.

So the premise here was let's measure the pesticides in these two different fruits and then scale that up and work out what that would look like in terms of a dose exposure in somebody eating normal amounts of these fruits. Yep. So when looking at bananas, they looked at 50 banana samples and residues were detected in 41 of these. A single pesticide residue was detected in 12 samples, but multiple residues were detected in 29 with two residues being found in six samples, three in 14 samples, four in five samples, five in three samples, and seven in one sample.

In the EU, maximum residue limits were exceeded in 14 samples for five different pesticides. In total, 16 different pesticide residues were recorded of which eight are unauthorized under EU legislation but represent 50% of all detected residues. So that's interesting. And are these food products that are making it to the EU?

I believe so, yeah. So even though they were being used for sale in the EU, potentially they may still contain pesticides in the food that wouldn't be allowed under EU agricultural laws. Triadimethon is one of these and this is the fungicide and was the most frequently detected compound was found to exceed the EU maximum residue level in six samples. It has been shown to cause developmental toxicity, reproductive impairment, neurological and cardiotoxicity and can induce oxidative stress.

Foranimal is a fungicide which was identified in 23 samples and was the second most detected pesticide but none of the samples breached the maximum residue levels for this. When looking at kiwi fruits, only 23 samples out of the 50 had detectable pesticide residues with 18 samples containing one residue and five containing multiple residues with four samples containing two and one sample containing three residues. 15 of these exceeded the EU maximum residue levels for nine pesticides with the most frequent being Acetimiprid in four samples and Clodinophob in four samples.

15 different pesticide residues were identified and four of these are unauthorised under EU regulations. And comparing this data to previous studies, there was an increase in detection frequency and the proportion of samples that exceeded the maximum residue levels of kiwi fruits from Turkey. So looking at both these fruits then, there were a reasonable number of detections of pesticide residues and it wasn't just one, it was actually multiple and they exceed some of the EU maximum residue levels. They then went on to look at the quality of the fruits.

So they were looking at this because multiple pesticide residues may adversely affect the quality due to additive or synergistic effects, even if individual pesticides are below maximum residue levels and

could pose concerns from a toxicological perspective. So 60% of bananas were found to exhibit satisfactory quality and 72% of kiwi fruit were found to be of satisfactory quality. So when we're talking about satisfactory quality here, we're not saying is this banana bruised. Yeah.

We are saying in terms of pesticide residue, does it meet the quality assurance level that we have set? Only 60% of bananas were meeting the EU quality assurance level for combined pesticide exposure. That feels bad, right? Yeah, it definitely does, yeah.

When looking at the hazard quotient, which is the exposure of concerned pesticide compared with acceptable daily intake and looking at the hazard index, which is the sum of the individual hazard quotient values for all detected pesticides, chronic health risks remain negligible despite 28 to 30% of samples exceeding the EU maximum residue levels. And I think this is critical. This is the real crux here, isn't it? We have set these limits.

Those limits are very conservative. And when you actually model the risk, the risk is deemed to be relatively low. And while the level of pesticides may exceed regulatory limits, the cumulative risks for adults and children were below critical thresholds indicating a negligible long-term health risk from consuming these fruits. Okay, but I am gonna put to you now a challenge.

Okay. These do make one big assumption. Yeah. They assume a typical daily intake of these fruits.

Yes. My daughter is obsessed with fruit and if allowed will eat five or six bananas a day. That's quite impressive. I feel like I might need to have her scale back, her banana consumption.

Just a smidge. It's a fair point though, isn't it? That this does assume normal intake. We've modeled these risks on a basis of an average daily intake.

Yes. And it's also not looking at the different kinds of pesticides you're encountering in a single sample as to whether they're working in a similar way, which could definitely increase your risk of long-term harms. That's fair. So cumulative exposure, potential accumulation.

I've just had another thought. Yeah. And you may or may not know the answer to this. Well, these sums done in isolation.

For example, I could model it and say, well, you only eat one banana a day. So if you eat one banana a day, you're going to be okay. But I also eat some potatoes and some cabbage and a kiwi fruit. Is my total dietary exposure being modeled?

See that, I don't think they did because that's quite hard to model. It'd be interesting to see whether looking at different diets, whether certain diets do increase your risk of exposure and whether you are meeting that requirement for long-term harm. And I guess this is the logic for having such conservative EU cut-offs, right? Well below the risk threshold because people don't just eat one banana and that's their daily food intake.

Yeah. This has definitely opened my eyes to pesticide exposure because again, you sort of put your head in the sand as a consumer, don't you? Especially in a country whereby we know we've got relatively good food regulation. Yeah.

Hopefully protecting me from significant pesticide exposure. But actually it has definitely got me thinking about it. Now we haven't actually touched on it, have we? But maybe we should a little bit.

There is obviously the organic food movement. Yeah. But there are also allowed pesticides within the organic food movement that are plant-derived pesticides. We were talking about the one that was used for fishing, for example.

Just because it's natural doesn't mean it's safe. Just because it's natural doesn't mean it's safe. Cyanide's natural. Yeah, exactly.

Definitely not good to put on your chips. So we've done flowers and we've done food. And now to keep the illiterations rolling, why don't we do something much more comfortable with me and that is forensics. So this next paper focuses on pesticide-related fatalities that were referred to diagnostic difficulty.

So this paper looked at 10 years worth of autopsy data that was evaluated for pesticide-related fatalities that were examined by the First Forensic Medicine Specialist Board of the Council of Forensic Medicine in Turkey. So they looked at the National Judiciary Informatic System to search for cases where the official cause of death was recorded as pesticide poisoning. 51 cases were analyzed in this data set with 57% of individuals being male and the mean age was 45.3 years. 31% of cases were younger than 30.

39% of cases were aged between 30 and 59 and 29% of cases were aged 60 or older. And this is a really interesting data set, isn't it? Because this wasn't a typical lab data set of the cases that a normal lab were encountering. This was a specific set of cases that were considered in inverted commas hard and sent to a specialist center.

I think it's a really good setup actually. Yeah. I really like the idea of a lab that only does the complicated stuff. I think you sometimes need that because you need people who are dealing with the complicated stuff day in and day out and they've got that expertise in it.

Whereas smaller labs might not have exposure to certain things so won't necessarily know whether that thing is relevant to a cause of death or not. Yeah, I think it's a really interesting model actually. So the youngest case involved accidental ingestion in a four-year-old and the oldest case involved an 88-year-old male who ingested the pesticide for the purposes of suicide. Two-thirds of individuals lived in urban areas with a third of the cases involving people living in rural regions.

And in terms of occupation, 27.5% of cases involved housewives, 24% involved farmers, 16% were students, 12% were unemployed and 22% were classed as other. These cases were distributed evenly throughout the year and 63% took place at residents with 86% taking place at an agricultural site. In 57% of cases, pesticides were identified at the death scene. In two cases, pesticides were not found at the scene and in 20 cases, no scene investigation was conducted.

21 different pesticide agents were identified from the scene investigation with 13 being fumigants or rodenticides. Herbicides were found in six cases. Five cases had carbamates. Four had a can of phosphates.

Four had pyrothiods. Four had fungicides. So why don't we drill into some of these a little bit? So fumigants were often talking about aluminium phosphide, aren't we?

Which releases phosphine gas. Sometimes used for, often for dealing with mammals. Moles is a famous one that it's used for, but other rats in buildings and so on and so forth. It doesn't actually say there, does it?

But rodenticides perhaps could include other rat poisons that we might be more familiar with, like warfarin. Carbamates and organophosphates are choline esterase inhibitors. We've talked about those. And pyrothroids are also compounds that target the nervous system and may prevent the closure of voltage-gated sodium channels.

In 51% of cases, individuals were hospitalized after exposure and CPR was attempted in a third of the cases. In 16% of cases, individuals were found dead at the scene without intervention. Where clinical

manifestations were recorded, the predominant features were CNS symptoms such as seizures, coma, or dizziness, which were found in 53% of cases. Muscarinic cholinergic signs were found in 31% of cases, which included pinpoint pupils, excessive salivation, sweating, GI symptoms, or respiratory distress.

Of the hospitalized cases, the duration of stay ranged from one to 42 days with the mean length of stay being 5.8 days. When looking at autopsy findings, foam in the mouth were found on exam in 12% of cases, liver histopathology revealed congestion, sinusoidal dilation, and inflammatory infiltration in 63% of cases, degenerative changes were found in 20% of cases, and necrotic or hemorrhagic alterations in 12% of cases. You may not know the answer to this, but the 12% that had foam in their mouth, were they the organophosphates and the carbamate group? I'm not sure off the top of my head.

In 31% of cases, a specific pesticide was detected. The most frequently confirmed agents were aluminium phosphide in six cases, methamyl in six cases, followed by malathion, zinc phosphide, phenoxaprop, PE phthal, endosulfan, and 2,4-D, and these were found each in one case. When looking at this toxicology, in 11 cases, the blood was positive pesticides, negative in 39, and not carried out in one case. Gastric contents were also tested and were positive pesticides in 15 cases, negative in 27, and not carried out in nine cases.

In urine toxicology, you confirmed the presence of pesticides in five cases. And this is actually really interesting, isn't it, when we look at these cases. Now, I guess, perhaps not every case had urine, but you've got more positivity here in blood than you have in urine. And in general, we sort of think of as urine as being the matrix that lets you detect more, not less, but the key matrix here was gastric content.

I don't know who's regularly testing gastric content. I know we don't tend to, but in this case, it seemed to be the best matrix for detecting them. Yeah, for sure. In terms of detections of alcohol along with these cases, 31 cases tested negative, six cases had an alcohol below 50 milligrams per deciliter, three cases had an alcohol between 50 and 100 milligrams per deciliter, three cases had an alcohol between 100 and 200 milligrams per deciliter, and one case had blood alcohol over 200 milligrams per deciliter.

So, possibly not too much to write home about there. Some cases had some reasonable ethanol, some didn't. Drug screening was also carried out in 46 of the cases with no illicit substances being detected in 42 cases. In the four that were positive, two were positive for cannabis, one was positive for MDMA, foria, and panaca, and one was positive for amphetamine.

That's an interesting range of compounds, isn't it? Cannabis, perhaps unsurprising, relatively commonly used. Yeah, that MDMA, foria, and panaca surprised me quite a bit. That is surprising, isn't it?

I'd love to know more about that specific case. And in conclusion with this data, pesticide-related deaths were rarely supported by a single source of data, with seed evidence being present in 29 of the cases, clinical information being important in 26, and toxicological information being found in 16 cases that supported pesticides. And I think that is really, really important here, isn't it? And there was a really nice Venn diagram in the paper that is hard to express in words on a podcast.

But it showed the overlap between seed evidence, toxicological evidence, and other supporting things. It showed actually that toxicology was really not that good at picking up pesticides. There were quite a few cases there whereby the clue wasn't the tox report. The clue was the circumstances and the seed evidence.

Now, the cynic amongst you could say, well, maybe it was coincidental. Maybe there was seed evidence and they've died of something else. But we need to drill into it a bit, don't we? Some of the compounds like, for example, the aluminium phosphide, absolute nightmare to detect post-mortem.

It is possible to detect phosphine gas in samples using headspace GCMS, but I'm not sure how many people are regularly doing headspace GCMS to look for phosphine gas. I can't imagine many. Pesticides in post-mortem cases are really quite interesting, aren't they? I mean, in this data set, there was a relatively low number because this was over what, a 10-year period?

Yeah. And they were only getting the complicated cases. So possibly there are cases out there that aren't being detected because they've got some other cause of death that's being ruled. But actually, they are relatively rare.

And as you say, labs really need a bit of expertise to feel familiar with these. But they do happen. I've had at least one or two pesticide cases and the clue was seen evidence rather than anything I picked up. I was able to confirm some of it on the mass spec retrospectively, but I wouldn't have found it if I didn't have the seen evidence that told me to go looking.

Yeah. It does make you wonder, doesn't it, how many cases are being missed? It is recognised that farmers and agricultural workers are at increased risk of suicide compared to the general population, believed to be due to the sort of loneliness of the work and the high pressure situation and so on. So possibly there are some cases that are falling through the net a little bit.

What surprised you most about these papers then? We've looked at three very different papers with a common theme being pesticides. I think the number of pesticides that are detected in food and in flowers in like a single sample, because I don't think you really think about lots of different forms of pesticides being used on the same thing. But obviously you're going to have different things you want to control for, like insects you want to control for other plant species and might be like invading your crops and things.

But I hadn't really fully appreciated that until we started reading for this episode. Yeah, definitely. I think it's definitely opened my eyes a bit. Has it changed how you approach your food?

Are you going to be washing your lettuce a little bit harder? I mean, probably not. I do wash my lettuce, but... Well, here is something we haven't talked about actually, that we've been talking about surface contamination.

Some of these are absorbed by the plants themselves, right? Yeah. So even surface washing may not remove them completely. But what about general risk?

Do you think we overestimate it or underestimate it? It's a good question. I think for a lot of the studies, we're looking at exposure limits for individual pesticides rather than pesticides that work in similar ways and cumulatively could pose a greater risk than we're anticipating. So I think it's a little bit of both that we both over and underestimate.

I think that's a very balanced answer. I think you're right. I think when you're looking at multiple pesticides, even if lots can be under the exposure limit, are they in combination posing health risk? I think the point though about how exposure limits are well under risk limits possibly protects you a little bit against that.

Yeah. But then again, as I said earlier, if we're assuming one banana is okay, but actually somebody's eating one banana, one kiwi fruit, a massive portion of cabbage, 12 potatoes, 12 potatoes? That's quite a lot of potatoes. I generally try to avoid carbohydrates, so I definitely don't eat 12 potatoes.

I'm not sure. That's maybe something to look further into as to whether there are cumulative exposures through dietary intake. But I think the thing that surprised me most about this really was that original Guardian article about flower workers and how people are working in these industries that, I don't know, you think of flowers and working in a florist. That sounds like, to me, an absolute dream job, right?

Yeah. I was assembling pretty bouquets and yet actually it could have a real dark, sinister chemical side that hadn't occurred to me at all. And the idea that some of these people were being exposed to significant concentrations of pesticides that could be having an impact on health actually just hadn't occurred to me.

Yeah, because when you think about pesticide exposure, you think of farmers and those working directly with the pesticide and directly with the crop rather than those downstream industries that are obviously going to be affected because they're handling those products day in and day out, possibly without having appropriate PPE to protect them from that sort of dermal exposure. Yeah, I think that's completely fair. I think so. And that's definitely where my mind has gone with this for sure.

It's definitely opened my eyes a bit to the pesticide world. Well, I hope you've enjoyed listening to us this week as we explore the world of pesticides and what that means to us as toxicologists. I have absolutely nothing but respect for our colleagues who do this regulatory work on flowers, on foods. It's really complex.

So absolute massive shout out to our colleagues that do that work. And also thank you again, Sandrine, for pointing us at the direction of that article because it really has opened our minds. I hope you've enjoyed listening to us and as always, please do give us a like, give us a follow, give us a share. We really appreciate it.

Hope you all have an amazing week and we'll see you next time. Bye.