Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Food Science

Progress towards the production of potatoes and cereals with low acrylamide-forming potential

Nigel G Halford, Sarah Raffan and Joseph Oddy

The presence of acrylamide in foods derived from grains, tubers, storage roots, beans and other crop products has become a difficult problem for the food industry. Here we review how acrylamide is formed predominantly from free asparagine and reducing sugars, the relationship between precursor concentration and acrylamide formation, and the challenge of complying with increasingly stringent regulations. Progress made in reducing acrylamide levels in foods is assessed, along with the difficulty of dealing with a raw material that may be highly variable due to plant responses to nutrition, disease, and cold storage. The potential for plant breeding and biotechnology to deliver low acrylamide varieties is assessed, in the context of a regulatory landscape covering acrylamide, crop biotechnology, and crop protection.

Address
Rothamsted Research, Harpenden AL5 2JQ, United Kingdom

Corresponding author: Nigel G Halford (nigel.halford@rothamsted.ac.uk)

Current Opinion in Food Science 2022, **47**:100887

This review comes from a themed issue on **Food Toxicology**

Edited by **Adriana Pavesi Arisseto-Bragotto**

https://doi.org/10.1016/j.cofs.2022.100887

Introduction

Acrylamide (C_3H_5NO) is a familiar chemical in biochemistry labs and in its polymeric form has a variety of industrial uses, including as a flocculant in wastewater and sewage treatment. While the polymeric form is nontoxic, the monomer is a potent neurotoxin, affects male reproduction, causes birth defects, and is carcinogenic in laboratory animals [1]. Formally, it is classed as a Group 2A carcinogen by the International Agency for Research on Cancer [2], as an 'extremely hazardous substance' in the United States and 'a serious health hazard with acute toxicity' in the European Union. It caused something of a seismic shock, therefore, when it was discovered in food [3].

Acrylamide is predominantly associated with fried, baked, roasted and toasted foods derived from plant grains, beans, tubers and storage roots, including bread (particularly when toasted), biscuits, breakfast cereals, potato crisps (US chips) and similar products produced from sweet potatoes or vegetables, French fries, roast potatoes and coffee [4]. It is not detectable in boiled foods and to our knowledge has never been found in raw crop products; it can therefore be classed as a processing contaminant.

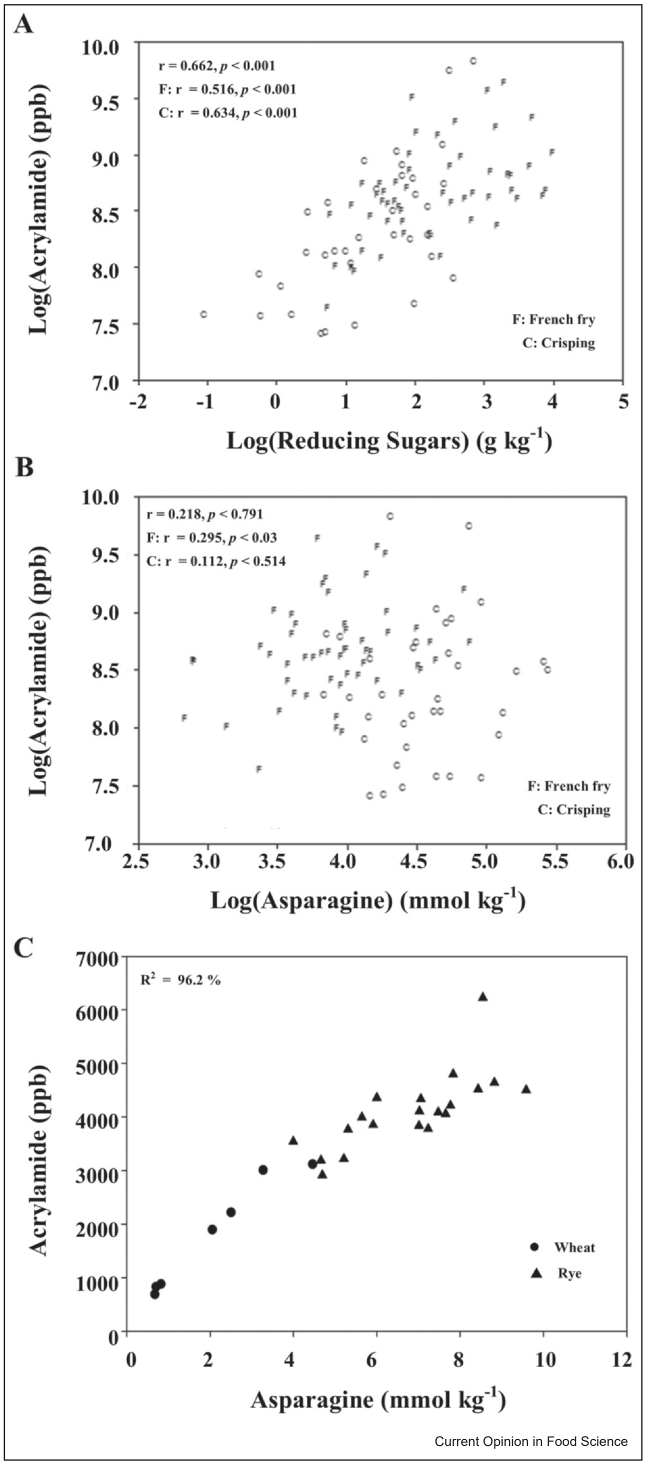
Acrylamide formation and its relationship with precursor concentration

Not long after acrylamide was discovered in food, it was demonstrated that it could form from free (soluble, non-protein) asparagine and reducing sugars, such as glucose, fructose and maltose, in the Maillard reaction [5,6]. We will not review the Maillard reaction in detail here: suffice to say that it is a complex series of reactions between amino groups, principally those of free amino acids, and reducing sugars. It is an important reaction for the food industry because it also produces the colour, flavour and aroma compounds that give fried, baked, toasted and roasted foods the characteristics that consumers expect.

It is important to note that other routes have been proposed for the formation of acrylamide, for example, with 3-aminopropionamide as a possible transient intermediate [7] or through pyrolysis of gluten [8] or oxidation of lipids [9]. Nevertheless, the Maillard reaction appears to be the predominant route, and free asparagine and reducing sugars can therefore be regarded as the precursors for acrylamide formation.

The ratio of free asparagine to reducing sugars in potatoes means that the concentrations of the latter usually determine the amount of acrylamide that forms in potato products [10–19] (Figure 1a). However, free asparagine concentration may contribute to the variance in acrylamide, particularly in varieties with relatively high reducing sugar concentrations [12,16–18] (Figure 1b). These varieties are generally those used for French fry rather than crisp production (denoted by F and C, respectively, in Figure 1, a and b), or those grown for the fresh food market. Modelling suggests that free asparagine concentration contributes to the variance in acrylamide formation when the ratio of free asparagine to reducing sugars is below approximately 2.3 [18]. Note that the data

Figure 1



Graphical representations of the relationships between precursor concentration and acrylamide formation in potato and wheat flour. **(A)** Relationship between reducing sugar concentration (g kg^{-1} on the log$_e$ scale) and acrylamide formation (ppb (μg kg^{-1}) on the log$_e$ scale) in potato flour heated to 160 °C for 20 min. Replotted from [16]. **(B)** Free asparagine concentration (mmol kg^{-1} on the log$_e$ scale) and acrylamide formation (ppb on the log$_e$ scale) in the same potato samples. Replotted from [16]. Points on graphs **A** and **B** from French fry varieties are denoted F, whereas those for crisping varieties are denoted C, the results for correlation (r) being given overall and then for types separately. **(C)** Free asparagine concentration and acrylamide formation in heated wheat and rye flour [20].

used for Figure 1a and 1b were obtained for potato flour after heating at 160 °C for 20 min. This method produces higher levels of acrylamide formation than are typically seen in commercial products, but gives a good, consistent indication of acrylamide-forming potential [17].

Glucose and fructose are the predominant reducing sugars in potato tubers, with very little maltose, and the ratio of glucose to fructose is also important. Both of these sugars contribute to the formation of acrylamide, but fructose has been shown to favour the production of acrylamide over colour compounds during the cooking of French fries, in comparison with glucose [21,22]. This is consistent with predictions obtained in a study modelling the kinetics of acrylamide formation in French fries [23], and blanching French fries to remove the soluble sugars and then adding glucose back to enable the Maillard reaction to proceed is a widely used acrylamide-mitigation measure [24•].

In wheat, rye and probably other cereals, the lower free asparagine concentration in the grain compared with potato tubers means that free asparagine rather than reducing sugar concentration determines acrylamide-forming potential (Figure 1c) (see [4,20] for a more detailed review).

The problem of regulatory compliance

There is no evidence that we are aware of that the discovery of acrylamide in food products has affected consumer preferences in any way. However, acrylamide in food has become a major regulatory compliance issue for the food industry. This is particularly true in the European Union, where the screening of products for acrylamide content has informed the development of the European Commission's risk-management measures. The data were analysed in the European Food Safety Authority (EFSA's) Scientific Panel on Contaminants in the Food Chain (CONTAM Panel) assessment of the risk posed by dietary acrylamide [1]. In that report, published in 2015, the panel expressed concern for the potential neoplastic (tumour-inducing) effects of dietary acrylamide, prompting the European Commission to introduce Commission Regulation (EU) 2017/2158 [25], which came into force in 2018.

Since the CONTAM report was published, a unique mutational 'signature' has been linked to acrylamide and its metabolite, glycidamide [26••]. Samples from 184 liver tumours and 217 tumours of other cancer types were found to carry this mutation and were considered likely to do so as a result of dietary or occupational exposure to acrylamide. Nevertheless, some researchers still question how great a risk is posed by dietary acrylamide: Eisenbrand [27•], for example, reviewed the available data on the genotoxicity of acrylamide and concluded that genotoxic effects occurred only at doses that were irrelevant to dietary exposure. However, this does not appear to have changed the opinion of the CONTAM Panel and the European Commission has a long record of acting on EFSA's advice alone.

Regulation (EU) 2017/2158 [25] set Benchmark Levels for acrylamide in different foods, including 500 parts per billion (ppb, μg kg^{-1}) for French fries, 750 ppb for potato crisps, 50 ppb for soft bread, 300 ppb for wheat-based breakfast cereals and 150 ppb for breakfast cereals made with other grains, 350 ppb for biscuits (150 ppb if they are for infants) and 40 ppb for cereal-based baby foods. The Benchmark Levels replaced Indicative Values, which had been in place since 2011, and for most products were lower. The justification given for this was that Indicative Values were 'triggers for investigation', whereas Benchmark Levels were 'performance indicators': there was little evidence that manufacturers had been able to reduce acrylamide levels in their products between 2011 and 2017. European Snacks Association data on acrylamide in potato crisps from 2002 to 2019, for example, showed a fall of 53% from 763 ppb in 2002 to 358 ppb in 2011, but then a flattening out, with the lowest mean so far being attained in 2018 at 353 ppb, scarcely lower than the 2011 figure [28••]. The progress made between 2002 and 2011 was due to measures such as improved control of cooking temperature and duration, blanching (removal of sugars and other soluble metabolites in hot water before frying), vacuum frying, control of moisture levels in the finished product, and the use of very low-sugar varieties, as well as postfrying quality control based on colour [24•]. Some manufacturers also introduced checks on potato sugar concentration at harvest, during storage and at the factory gate.

By 2019, 7.75% of crisp samples still remained above the Benchmark Value of 750 ppb, while pronounced geographical and seasonal effects on acrylamide levels meant that there were striking differences between regions and a strong trend of higher acrylamide levels from November to May (Figure 2), arising from the behaviour of potatoes in cold storage [28••]. European potatoes are harvested between July and October and for the rest of the year are used from storage. Potatoes in storage are prone to cold and senescent sweetening, both of which bring about an increase in glucose and fructose concentrations associated with vacuolar invertase (VInv) activity [29–31]. Senescent sweetening is also driven by the breakdown of starch through the actions of phosphorylase L (PhL) and starch-associated R1 (R1). Manufacturers are careful to use potatoes only within their optimum storage window, which differs between varieties, and the temperature and other conditions of commercial potato stores are very carefully controlled. Nevertheless, the proportion of samples exceeding the 750 ppb mark in the 2017–2019 period was almost 18% in southern Europe in January and above 10% in every region for some of the year (Figure 2). Overall, there is little to suggest that acrylamide levels in potato crisps could be kept below the current Benchmark Level all of the time.

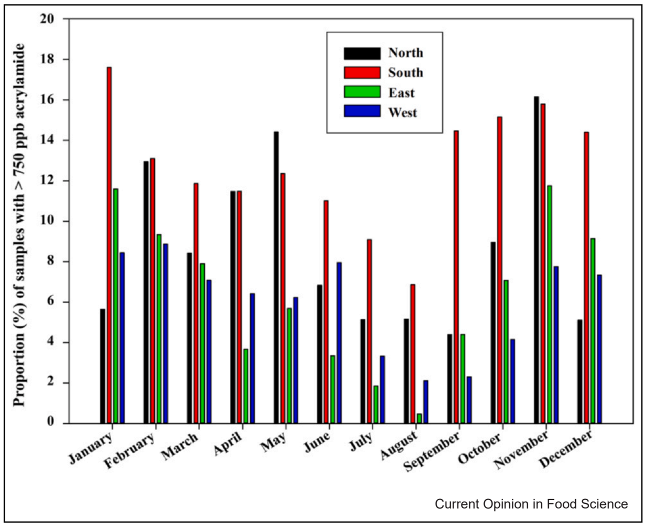
Data in the public domain on acrylamide levels in cereal products are relatively sparse, but a recent study in Spain found that 15% of breakfast cereals contained acrylamide above the Benchmark Level [32••]. The European Commission is currently considering replacing Benchmark Levels with Maximum Levels [33•], to be set in accordance with Regulation (EEC) No 315/93 [34]. It would be illegal to sell a product containing acrylamide above the Maximum Level for that product type, yet the intention appears to be to set Maximum Levels at or below the current Benchmark Levels. This would have serious repercussions for the food industry, with manufacturers and retailers possibly facing the prospect of having to deal with product recalls or even prosecutions.

Crop-management strategies

Different fertilisation regimes have been known to affect potato tuber composition since the 1990s [35] and the discovery of acrylamide in potato products led to many more studies being conducted, particularly on the effect of nitrogen and, to a lesser extent, sulphur fertilisation [10,15,36]. We will not review the results of those studies in detail here because we have done so previously [4], but summarise by saying that the effects are complex, with nitrogen application generally increasing the acrylamide-forming potential of French fry varieties while having no effect on crisping varieties, but with different varieties within type also showing markedly different responses. Sulphur application can mitigate the effect of high nitrogen application but only in some varieties. The complexity of these responses means that general advice has never been issued on the optimal levels of nitrogen and sulphur application to potatoes to address the acrylamide issue.

Drought stress has also been shown to initiate complex responses in potatoes, with severe drought stress causing a big increase in free asparagine concentration that was not observed under mild stress [37] and, as with responses to nutrition, different varieties responding in different ways, indicating that there is no single, unifying potato drought-stress response. One amino acid that was shown to increase substantially (15-fold) under mild stress conditions was free proline and this did

Figure 2



Current Opinion in Food Science

Proportion (%) of samples of potato crisps produced by manufacturers affiliated to the European Snacks Association from 2017 to 2019 with more than 750 ppb acrylamide, shown for each month and separated into geographic regions of Europe (north, south, east and west). Replotted from [28••].

correlate negatively with acrylamide formation [37], consistent with proline being shown to inhibit acrylamide formation in model systems [38].

In contrast to potato, the responses of wheat and barley to nitrogen and sulphur are clear and well-established, with nitrogen causing a rise in free asparagine concentration in the grain [39–41] and sulphur having the opposite effect [42–46]. Sulphur deficiency in particular causes a huge increase in free asparagine concentration in wheat grain and we recommend that nitrogen fertiliser should not be applied to excess and should always be accompanied by sufficient sulphur. Both nitrogen and sulphur application also affect rye, but the effect of sulphur under field conditions is much less severe [47], perhaps because rye is better at scavenging available sulphur even when it is scarce, but also because rye appears to respond differently, with wheat and barley using free asparagine as a nitrogen store when sulphur is scarce in a way that rye does not.

Free asparagine concentration in wheat grain can increase in response to biotic as well as abiotic stress, and good disease control has been shown to be another important acrylamide-mitigation measure [41,48].

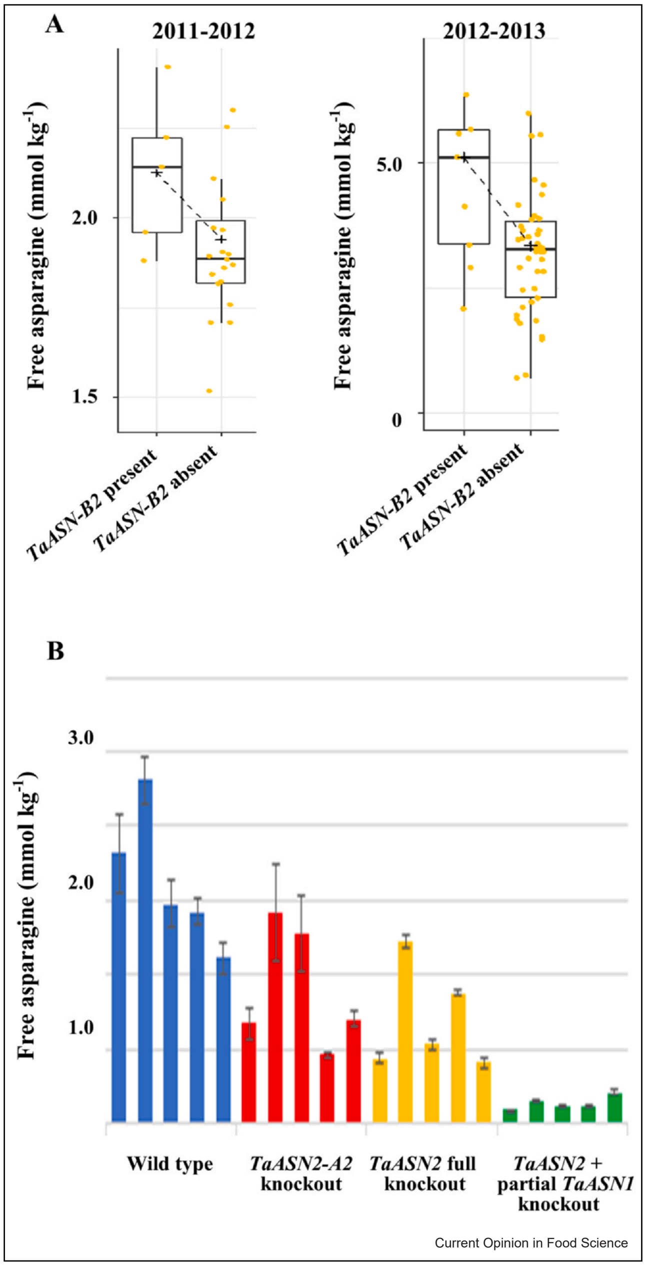
Genetic and biotech approaches

Since crop-management and food-processing strategies to reduce acrylamide formation in foods have already been implemented [24•], further gains from those approaches may be difficult to achieve. This makes it important that plant breeders engage on the acrylamide issue and produce new varieties with reduced acrylamide-forming potential. This would enable food manufacturers to comply with tightening regulations on acrylamide without costly changes to production lines or damaging reductions in product quality. In potato, breeders have been selecting for low reducing sugar concentration for several decades to enable crisp manufacturers to produce crisps with an even, golden colour. Further reductions by conventional breeding may be difficult to achieve, but the genetic control of free asparagine and reducing sugar concentration during storage may be viable additional targets. Indeed, both have been the target for biotechnological interventions already: low acrylamide genetically modified (GM) potato varieties, Innate® and Innate® Generation 2 [49,50], have reduced expression of *StASN1*, one of the two asparagine synthetase genes of potato, specifically in the tubers [51]. These varieties were developed by the Simplot Company of Boise, Idaho, and have been on the market in the United States and Canada for several years. Both also have decreased expression of genes *PhL* (starch phosphorylase L) and *R1* to slow down starch breakdown during storage, while Innate® Generation 2 also has reduced expression of a vacuolar invertase gene (*VInv*), so is less prone to cold and senescent sweetening. The *VInv* gene has also been targeted using the transcription activator-like effector nucleases (TALENs) genome editing technique [52], resulting in potatoes with undetectable levels of reducing sugars and crisps with much lower levels of acrylamide than controls. However, varieties carrying this trait have not been commercialised yet.

Several studies have shown large varietal differences in the free asparagine content of wheat grain [45,46,48,53–56]. In field trials of winter wheat varieties in the United Kingdom, for example, free asparagine concentration ranged from 0.71 to 11.29 mmol kg^{-1} [46]. That study also showed that, while varietal rankings changed from one year to the next due to environmental factors, it was possible to identify some varieties that were consistently low. The problem for food businesses is that by the time multiyear studies have established whether a variety is low or high for free asparagine, new varieties are coming onto the market. We would support free asparagine concentration being measured during variety development, but this is not standard practice at present. Varietal differences in and environmental influences on free asparagine concentration have also been revealed for rye, maize and oats [47,56,57].

The wide range in free asparagine concentration in the grain of different varieties suggests that it should be possible to select for the low asparagine trait, and free

Figure 3



Current Opinion in Food Science

Evidence for reduced free asparagine in wheat grain due to natural and induced mutations in *TaASN2* genes. **(A).** Concentrations (back-transformed data) of free asparagine in the grain of different varieties of wheat in which the *TaASN-B2* gene was either present or absent, grown in field trials with plentiful sulphur as well as nitrogen in the United Kingdom in 2011–2012 and 2012–2013. Crosses indicate the means, while boxes show the interquartile range and median, and whiskers show the smallest and largest value within 1.5 times the interquartile range above and below the 75th and 25th percentile. Replotted from [61]. **(B).** Free asparagine concentrations in the grain of wheat (*Triticum aestivum*) cv. Cadenza (wild type) and plants in which the *TaASN-A2* gene, all of the *TaASN2* genes and all of the *TaASN2* genes plus one *TaASN1* gene were 'knocked out' using CRISPR/Cas9. The plants were from the T2 generation and free asparagine concentrations are shown for five individual seeds from each plant. Replotted from [68••].

asparagine concentration has been shown to have moderate heritability in some studies (e.g. [58,59••]). There is also little evidence to suggest that breeding for low grain asparagine concentration would have a negative effect on other agronomic traits [59••,60].

The breeding of low asparagine wheat or other cereals would be greatly facilitated by the development of genetic resources associated with the trait. One such resource for wheat arises from the natural deletion of an asparagine synthetase gene [61]. Wheat has five asparagine synthetase genes on each genome: *TaASN1*, *TaASN2*, *TaASN3.1*, *TaASN3.2* and *TaASN4* [62,63•], with the *TaASN2* gene highly and specifically expressed in the embryo and endosperm of the grain [64,65]. In some varieties, the *TaASN2* gene on the B genome (*TaASN-B2*) is missing, and this has been shown to affect the free asparagine content of grain in two different field trials (Figure 3a) [61]. However, to date, this represents the only multienvironment quantitative trait locus (QTL) for low asparagine because other QTL for the trait have not yet been verified across more than one environment [66,67].

As with potato, biotechnology could have an important part to play in developing wheat and other cereals with substantially reduced acrylamide-forming potential. For example, genome editing using the clustered regularly interspaced palindromic repeats/CRISPR-associated protein 9 (CRISPR/Cas9) system has been used to 'edit' the *TaASN2* genes of wheat, producing partial and total 'knockouts' of the gene [68••], reducing free asparagine concentration by up to 90% in glasshouse experiments (Figure 3B). The line showing that level of reduction turned out to have an additional partial 'knockout' of the *TaASN1* gene (unpublished data). These edited lines grew normally under glass, except that in one generation, they showed poor germination, something that could be rescued by applying asparagine solution to the soil [68••]. Some of the lines also showed an increase in grain weight, and this requires further investigation. A field trial of the partial and full *TaASN2* 'knockout' lines was sown in October 2021 and the plants germinated well (unpublished data).

The fact that there is a single copy of each asparagine synthetase gene per genome in wheat lends itself to chemical mutagenesis as well as genome editing, and wheat plants with ethyl methanesulfonate induced null *TaASN-A2* alleles have already been studied in a field trial [69••]. The plants showed reductions of between 9% and 34% in free asparagine concentration in the grain with no apparent effects on yield or quality traits.

Similar asparagine synthetase gene families to that of wheat are present in the other members of the Triticea tribe, including barley and rye, but sorghum, maize and rice have fewer genes and all lack the *TaASN2* gene [63•]. Indeed, rice has only two asparagine synthetase genes, *OsASN1* and *OsASN2* (equivalent to *TaASN4* and *TaASN3*, respectively) and lines lacking a functional *OsASN1* gene showed effects on plant height, root length and tiller number [70••].

Conclusions

The presence of acrylamide in popular foods is an increasingly difficult regulatory compliance issue for the European food industry, whether located in the European Union or selling into that market. It is clear that the European Commission expects to see acrylamide levels in food decrease in response to its risk-management measures and if these expectations are not met, it intends to ramp up its measures, up to and including the introduction of Maximum Levels for some products. Regulators in other parts of the world may well follow suit. Some sectors of the European food industry were able to show impressive reductions in acrylamide in the first decade after acrylamide was discovered in food [28••], mainly arising from changes to manufacturing processes and crop management or better quality control. Those improvements have slowed, and further substantial reductions from those approaches may be difficult to achieve. Food manufacturers are, therefore, looking to plant breeders to develop varieties with lower acrylamide-forming potential.

Plant breeding is a slow process, but it is now 20 years since acrylamide was discovered in food and the food industry may well ask why such varieties are not yet available. Clearly, reducing sugars and asparagine are important metabolites and decreasing their concentrations in crop products may not be easy. In addition, plant breeders often attach higher priority to issues that impact farmers directly over those that affect the food industry. That may change if farmers find that their crops become unsuitable for some high-value end uses, but plant breeders need to be preparing now for future regulations on acrylamide.

Genetic modification and genome editing could speed up the process of developing low acrylamide varieties and take acrylamide-forming potential well below the range achievable by conventional breeding. Indeed, as we have discussed, low acrylamide GM potato varieties are already on the market in the United States [49–51]. There is no prospect at all of similar varieties being developed for cultivation in the European Union, however, because the EU approval process for GM varieties is so dysfunctional that no one even attempts to negotiate it [71]. The situation for genome edited crops is no different because under current EU regulations, genome edited crops have to be treated as if they were GM, and edited genes as if they were transgenes. This is an example of how one set of regulations in the European Union may hinder attempts to comply with another set of regulations. The UK government on the other hand has already revised its regulations on field trials of genome edited crops and the Genetic Technologies (Precision Breeding) bill, which establishes a new regulatory framework for the commercialisation of genome edited crops, is currently being considered by the UK parliament.

Another example of EU regulations not being joined up is the refusal in 2019 of the European Commission's Standing Committee on Plants, Animals, Food and Feed to renew the licence for chlorpropham (CIPC, isopropyl (3-chlorophenyl)carbamate)), the potato-storage industry's preferred sprout suppressant. Although there are some alternatives [72,28••], the loss of chlorpropham has certainly made potato storage more difficult and may lead to stores being kept at colder temperatures, increasing the risk of cold sweetening and exacerbating the acrylamide problem. The range of crop-protection chemistry available to farmers in the European Union has also been reduced [73]. There are complex arguments for and against this policy that are beyond the scope of this review, but good phytosanitary practice is included as a compulsory acrylamide-mitigation measure in Commission Regulation (EU) 2017/2158 [25], yet this does not seem to have been considered at all when assessing the impact of withdrawing authorisation for important pesticides and fungicides.

Conflict of interest statement

The authors declare no conflict of interest. The funders listed below had no role in the writing of this review, or in the decision to publish.

Acknowledgements

SR is supported by a Biotechnology and Biological Sciences Research Council (BBSRC) Super Follow-on Fund grant (BB/T017007/1), with partners: University of Bristol, AHDB, KWS UK Ltd, Saaten Union UK Ltd, RAGT Seeds Ltd, Syngenta UK Ltd, and Limagrain UK Ltd. JO is supported by a BBSRC Collaborative Training Partnership Studentship (BB/T50838X/1) with partners: University of Reading and Mondelez UK R &D Ltd. NGH is supported at Rothamsted Research by the BBSRC via the Designing Future Wheat Programme (BB/P016855/1). BBSRC is part of UK Research and Innovation.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. CONTAM Panel (European Food Safety Authority Panel on Contaminants in the Food Chain): **Scientific opinion on acrylamide in food**. *EFSA J* 2015, **13**:4104.

2. IARC (International Agency for Research on Cancer): **IARC Monographs on the Evaluation of Carcinogenic Risks to Humans Vol 60. Some Industrial Chemicals**. International Agency for Research on Cancer (IARC); 1994.

3. Tareke E, Rydberg P, Karlsson P, Eriksson S, Törnqvist M: **Analysis of acrylamide, a carcinogen formed in heated foodstuffs**. *J Agric Food Chem* 2002, **50**:4998-5006.

4. Raffan S, Halford NG: **Acrylamide in food: progress in and prospects for genetic and agronomic solutions**. *Ann Appl Biol* 2019, **175**:259-281.

5. Mottram DS, Wedzicha BL, Dodson AT: **Acrylamide is formed in the Maillard reaction**. *Nature* 2002, **419**:448-449.

6. Stadler RH, Blank I, Varga N, Robert F, Hau J, Guy PA, Robert M-C, Riediker S: **Acrylamide from Maillard reaction products**. *Nature* 2002, **419**:449-450.

7. Granvogl M, Schieberle P: **Thermally generated 3-aminopropionamide as a transient intermediate in the formation of acrylamide**. *J Agric Food Chem* 2006, **54**:5933-5938.

8. Claus A, Weisz GM, Schieber A, Carle R: **Pyrolytic acrylamide formation from purified wheat gluten and gluten-supplemented wheat bread rolls**. *Mol Nutr Food Res* 2006, **50**:87-93.

9. Kuek L, Tarmizi AHA, Razak RAA, Jinap S, Norliza S, Sanny M: **Contribution of lipid towards acrylamide formation during intermittent frying of French fries**. *Food Control* 2020, **118**:107430.

10. Amrein TM, Bachmann S, Noti A, Biedermann M, Barbosa MF, Biedermann-Brem S, Grob K, Keiser A, Realini P, Escher F, Amadò R: **Potential of acrylamide formation, sugars, and free asparagine in potatoes: a comparison of cultivars and farming systems**. *J Agric Food Chem* 2003, **51**:5556-5560.

11. Amrein TM, Schönbächler B, Rohner F, Lukac H, Schneider H, Keiser A, Escher F, Amadò R: **Potential for acrylamide formation in potatoes: data from the 2003 harvest**. *Eur Food Res Technol* 2004, **219**:572-578.

12. Becalski A, Lau BP-Y, Lewis D, Seaman SW, Hayward S, Sahagian M, Ramesh M, Leclerc Y: **Acrylamide in French fries: influence of free amino acids and sugars**. *J Agric Food Chem* 2004, **52**:3801-3806.

13. Burch RS, Trzesicka A, Clarke M, Elmore JS, Briddon A, Matthews W, Webber N: **The effects of low-temperature potato storage and washing and soaking pre-treatments on the acrylamide content of French fries**. *J Sci Food Agric* 2008, **88**:989-995.

14. De Wilde T, De Meulenaer B, Mestdagh F, Govaert Y, Vandeburie S, Ooghe W, Fraselle S, Demeulemeester K, van Peteghem C, Calus A, Degroodt JM, Verhe RA: **Influence of storage practices on acrylamide formation during potato frying**. *J Agric Food Chem* 2005, **53**:6550-6557.

15. De Wilde T, De Meulenaer B, Mestdagh F, Govaert Y, Vandeburie S, Ooghe W, Fraselle S, Demeulemeester K, van Peteghem C, Calus A, Degroodt JM, Verhe RA: **Influence of fertilization on acrylamide formation during frying of potatoes harvested in 2003**. *J Agric Food Chem* 2006, **54**:404-408.

16. Halford NG, Muttucumaru N, Powers SJ, Gillatt PN, Hartley L, Elmore JS, Mottram DS: **Concentrations of free amino acids and sugars in nine potato varieties: effects of storage and relationship with acrylamide formation**. *J Agric Food Chem* 2012, **60**:12044-12055.

17. Muttucumaru N, Powers SJ, Briddon A, Elmore JS, Mottram DS, Halford NG: **Evidence for the complex relationship between the concentrations of free amino acids, sugars and acrylamide-forming potential in potato**. *Ann Appl Biol* 2014, **164**:286-300.

18. Muttucumaru N, Powers SJ, Elmore JS, Dodson A, Briddon A, Mottram DS, Halford NG: **Acrylamide-forming potential of potatoes grown at different locations, and the ratio of free asparagine to reducing sugars at which free asparagine becomes a limiting factor for acrylamide formation**. *Food Chem* 2017, **220**:76-86.

19. Elmore JS, Briddon A, Dodson AT, Muttucumaru N, Halford NG, Mottram DS: **Acrylamide in potato crisps prepared from 20 UK-grown varieties: effects of variety and tuber storage time**. *Food Chem* 2015, **182**:1-8.

20. Curtis TY, Postles J, Halford NG: **Reducing the potential for processing contaminant formation in cereal products**. *J Cereal Sci* 2014, **59**:382-392.

21. Mestdagh F, De Wilde T, Castelein P, Németh O, van Peteghem C, De, Meulenaer B: **Impact of the reducing sugars on the relationship between acrylamide and Maillard browning in French fries**. *Eur Food Res Technol* 2008, **227**:69-76.

22. Higley J, Kim J-Y, Huber KC, Smith G: **Added versus accumulated sugars on colour development and acrylamide formation in french-fried potato strips**. *J Agric Food Chem* 2012, **60**:8763-8771.

23. Parker JK, Balagiannis DP, Higley J, Smith G, Wedzicha BL, Mottram DS: **Kinetic model for the formation of acrylamide during the finish-frying of commercial French fries**. *J Agric Food Chem* 2012, **60**:9321-9331.

24. • FoodDrinkEurope: Acrylamide Toolbox 2019. FoodDrinkEurope; 2019.

This is the regularly updated compendium of acrylamide mitigation measures produced by FoodDrinkEurope, an EU food industry federation

25. European Commission: **Commission Regulation (EU) 2017/2158 of 20 November 2017 Establishing Mitigation Measures and Benchmark Levels for the Reduction of the Presence of Acrylamide in Food**. European Commission; 2017.

26. •• Zhivagui M, Ng AWT, Ardin M, Churchwell MI, Pandey M, Renard C, Villar S, Cahais V, Robitaille A, Bouaoun L, Heguy A, Guyton KZ, Stampfer MR, McKay J, Hollstein M, Olivier M, Rozen SG, Beland FA, Korenjak M, Zavadil J: **Experimental and pan-cancer genome analyses reveal widespread contribution of acrylamide exposure to carcinogenesis in humans**. *Genome Res* 2019, **29**:521-531.

This reference describes the identification of a unique mutational 'signature' linked to acrylamide and its metabolite, glycidamide, most likely arising from dietary exposure.

27. • Eisenbrand G: **Revisiting the evidence for genotoxicity of acrylamide (AA), key to risk assessment of dietary AA exposure**. *Arch Toxicol* 2020, **94**:2939-2950.

This reference is testament to the continuing lack of agreement on the carcinogenic properties of acrylamide, concluding that compelling evidence for a genotoxic mode of action is lacking.

28. •• Powers SJ, Mottram DS, Curtis A, Halford NG: **Progress on reducing acrylamide levels in potato crisps in Europe, 2002 to 2019**. *Food Addit Contam Part A* 2021, **38**:782-806.

This reference reported the analysis of data on acrylamide in potato crisps in Europe from 2002 to 2019, showing a reduction from 2002 to 2011 followed by a plateauing, with pronounced seasonal and geographical effects exacerbating the problem of compliance with EU Benchmark Levels.

29. Clasen BM, Stoddard TJ, Luo S, Demorest ZL, Li J, Cedrone F, Tibebu R, Daviso S, Ray EE, Daulhac A, Coffman A, Yabandith A, Retterath A, Haun W, Baltes NJ, Mathis L, Voytas DF, Zhang F: **Improving cold storage and processing traits in potato through targeted gene knockout**. *Plant Biotech J* 2016, **14**:169-176.

30. Zhu XB, Richael C, Chamberlain P, Busse JS, Bussan AJ, Jiang JM, Bethke PC: **Vacuolar invertase gene silencing in potato (*Solanum tuberosum* L.) improves processing quality by decreasing the frequency of sugar-end defects.** *PLoS One* 2014, **9**:e93381.

31. Wiberley-Bradford AE, Bethke PC: **Suppression of the vacuolar invertase gene delays senescent sweetening in chipping potatoes**. *J Sci Food Agric* 2018, **98**:354-360.

32. •• Mesías M, Sáez-Escudero L, Morales FJ, Delgado-Andrad C: **Reassessment of acrylamide content in breakfast cereals. Evolution of the Spanish market from 2006 to 2018**. *Food Control* 2019, **105**:94-101.

This reference reviews progress in reducing acrylamide levels in breakfast cereals in Spain over a 12-year period, finding that 15 % of breakfast cereals contained acrylamide above the EU Benchmark Level.

33. European Commission: **Food Safety: Acrylamide**; 2021, URL
• ⟨https://ec.europa.eu/food/safety/chemical-safety/contaminants/catalogue/acrylamide_en⟩ (Accessed 21st March 2022).

This reference is a web page maintained by the European Commission giving information on acrylamide. It states that the setting of Maximum Levels for acrylamide in certain foods is currently under consideration.

34. European Commission: **Council Regulation (EEC) No 315/93 of 8 February 1993 laying down Community Procedures for Contaminants in Food**. European Commission; 1993.

35. Roe MA, Faulk RM, Belsten JL: **Role of reducing sugars and amino acids in fry colour of chips from potatoes grown under different nitrogen regimes**. *J Sci Food Agric* 1990, **52**:207-214.

36. Muttucumaru N, Powers SJ, Elmore JS, Mottram DS, Halford NG: **Effects of nitrogen and sulphur fertilization on free amino acids, sugars and acrylamide-forming potential in potato**. *J Agric Food Chem* 2013, **61**:6734-6742.

37. Muttucumaru N, Powers SJ, Elmore JS, Mottram DS, Halford NG: **Effects of water availability on free amino acids, sugars and acrylamide-forming potential in potato**. *J Agric Food Chem* 2015, **63**:2566-2575.

38. Koutsidis G, Simons SPJ, Thong YH, Haldoupis Y, Mojica-Lazaro J, Wedzicha BL, Mottram DS: **Investigations on the effect of amino acids on acrylamide, pyrazines, and Michael addition products in model systems**. *J Agric Food Chem* 2009, **57**:9011-9015.

39. Winkler U, Schön WJ: **Amino acid composition of the kernel proteins in barley resulting from nitrogen fertilization at different stages of development**. *J Agron Crop Sci* 1980, **149**:503-512.

40. Claus A, Schreiter P, Weber A, Graeff S, Herrmann W, Claupein W, Schieber A, Carle R: **Influence of agronomic factors and extraction rate on the acrylamide contents in yeast-leavened breads**. *J Agric Food Chem* 2006, **54**:8968-8976.

41. Martinek P, Klem K, Vanova M, Bartackova V, Vecerkova L, Bucher P, Hajslova J: **Effects of nitrogen nutrition, fungicide treatment and wheat genotype on free asparagine and reducing sugars content as precursors of acrylamide formation in bread**. *Plant Soil Environ* 2009, **55**:187-195.

42. Shewry PR, Franklin J, Parmar S, Smith SJ, Miflin BJ: **The effects of sulphur starvation on the amino acid and protein compositions of barley grain**. *J Cereal Sci* 1983, **1**:21-31.

43. Muttucumaru N, Halford NG, Elmore JS, Dodson AT, Parry M, Shewry PR, Mottram DS: **The formation of high levels of acrylamide during the processing of flour derived from sulfate-deprived wheat**. *J Agric Food Chem* 2006, **54**:8951-8955.

44. Granvogl M, Wieser H, Koehler P, Von Tucher S, Schieberle P: **Influence of sulphur fertilization on the amounts of free amino acids in wheat. Correlation with baking properties as well as with 3-aminopropionamide and acrylamide generation during baking**. *J Agric Food Chem* 2007, **55**:4271-4277.

45. Curtis TY, Muttucumaru N, Shewry PR, Parry MA, Powers SJ, Elmore JS, Mottram DS, Hook S, Halford NG: **Effects of genotype and environment on free amino acid levels in wheat grain: implications for acrylamide formation during processing**. *J Agric Food Chem* 2009, **57**:1013-1021.

46. Curtis TY, Powers SJ, Wang R, Halford NG: **Effects of variety, year of cultivation and sulphur supply on the accumulation of free asparagine in the grain of commercial wheat varieties**. *Food Chem* 2018, **239**:304-313.

47. Postles J, Powers S, Elmore JS, Mottram DS, Halford NG: **Effects of variety and nutrient availability on the acrylamide forming potential of rye grain**. *J Cereal Sci* 2013, **57**:463-470.

48. Curtis TY, Powers SJ, Halford NG: **Effects of fungicide treatment on free amino acid concentration and acrylamide-forming potential in wheat**. *J Agric Food Chem* 2016, **64**:9689-9696.

49. USDA-APHIS: **J.R. Simplot Company Petition (13-022-01p) for Determination of Non-regulated Status of Low Acrylamide Potential and Reduced Black Spot Bruise Potato Events F10, F37, E12, E24, J3, J78, G11, H37, and H50: Plant Pest Risk Assessment**. USDA-APHIS; 2013.

50. USDA-APHIS: **J.R. Simplot Company Petition (14-093-01p) for Determination of Nonregulated Status for Innate^{TM} Potatoes with Late Blight Resistance, Low Acrylamide Potential, Reduced Black Spot and Lowered Reducing Sugars: Russet Burbank Event W8**. USDA-APHIS; 2014.

51. Chawla R, Shakya R, Rommens CM: **Tuber-specific silencing of *asparagine synthetase-1* reduces the acrylamide-forming potential of potatoes grown in the field without affecting tuber shape and yield**. *Plant Biotechnol J* 2012, **10**:913-924.

52. Clasen BM, Stoddard TJ, Luo S, Demorest ZL, Li J, Cedrone F, Tibebu R, Daviso S, Ray EE, Daulhac A, Coffman A, Yabandith A, Retterath A, Haun W, Baltes NJ, Mathis L, Voytas DF, Zhang F: **Improving cold storage and processing traits in potato through targeted gene knockout**. *Plant Biotechnol J* 2016, **14**:169-176.

53. Taeymans D, Wood J, Ashby P, Blank I, Studer A, Stadler RH, Gonde P, Van Eijck P, Lalljie S, Lingnert H, Lindblom M, Matissek R, Muller D, Tallmadge D, O'Brien J, Thompson S, Silvani D, Whitmore T: **A review of acrylamide: an industry perspective on research, analysis, formation and control**. *Crit Rev Food Sci Nutr* 2004, **44**:323-347.

54. Claus A, Schreiter P, Weber A, Graeff S, Herrmann W, Claupein W, Schieber A, Carle R: **Influence of agronomic factors and extraction rate on the acrylamide contents in yeast-leavened breads**. *J Agric Food Chem* 2006, **54**:8968-8976.

55. Corol DI, Ravel C, Rakszegi M, Charmet G, Bedo Z, Beale MH, Shewry PR, Ward JL: **^{1}H NMR screening for the high-throughput determination of genotype and environmental effects on the content of asparagine in wheat grain.** *Plant Biotech J* 2016, **14**:128-139.

56. Žilić S, Dodig D, Basić Z, Vančetović J, Titan P, Đurić N, Tolimir N: **Free asparagine and sugars profile of cereal species: the potential of cereals for acrylamide formation in foods**. *Food Addit Contam Part A* 2017, **34**:705-713.

57. Curtis TY, Powers SJ, Balagiannis D, Elmore JS, Mottram DS, Parry MAJ, Raksegi M, Bedő Z, Shewry PR, Halford NG: **Free amino acids and sugars in rye grain: implications for acrylamide formation**. *J Agric Food Chem* 2010, **58**:1959-1969.

58. Rapp M, Schwadorf K, Leiser WL, Würschum T, Longin CF: **Assessing the variation and genetic architecture of asparagine content in wheat: what can plant breeding contribute to a reduction in the acrylamide precursor?** *Theor Appl Genet* 2018, **131**:2427-2437.

59. Oddy J, Raffan S, Wilkinson M, Elmore S, Halford NG:
•• **Understanding the relationships between free asparagine in grain and other traits to breed low-asparagine wheat**. *Plants* 2022, **11**:669.

This reference reviews the evidence that reducing free asparagine concentration in wheat grain could be achieved without compromising other traits. Includes all heritability estimates for asparagine concentration.

60. Malunga LN, Ames NP, Masatcioglu MT, Khorshidi AS, Thandapilly SJ, Cuthbert RD, Sopiwnyk E, Scanlon MG: **Free asparagine concentrations in Canadian hard red spring wheat cultivars**. *Can J Plant Sci* 2019, **99**:338-347.

61. Oddy J, Alárcon-Reverte R, Wilkinson M, Ravet K, Raffan S, Minter
•• A, Mead A, Elmore JS, Moreira de Almeida I, Cryer NC, Halford NG, Pearce S: **Reduced free asparagine in wheat grain resulting from a natural deletion of *TaASN-B2*: investigating and exploiting diversity in the asparagine synthetase gene family to improve wheat quality**. *BMC Plant Biol* 2021, **21**:302.

This reference describes a natural deletion that is responsible for the lack of a *TaASN-B2* asparagine synthetase gene in some wheat varieties, and shows its distribution in a range of UK and global wheat varieties and related species.

62. Xu H, Curtis TY, Powers SJ, Raffan S, Gao R, Huang J, Heiner M, Gilbert D, Halford NG: **Genomic, biochemical and modelling analyses of asparagine synthetases from wheat**. *Front Plant Sci* 2018, **8**:2237.

63. Raffan S, Halford NG: **Phylogenetic analysis of cereal
• asparagine synthetase genes**. *Ann Appl Biol* 2021, **178**:6-22.

This reference describes the asparagine synthetase gene families from a range of cereal species, showing that *TaASN2*, the most highly expressed asparagine synthetase gene in wheat grain, has no equivalent in maize, rice or sorghum.

64. Gao R, Curtis TY, Powers SJ, Xu H, Huang J, Halford NG: **Food safety: structure and expression of the asparagine synthetase gene family of wheat**. *J Cereal Sci* 2016, **68**:122-131.
65. Curtis TY, Raffan S, Wan Y, King R, Gonzalez-Uriarte A, Halford NG: **Contrasting gene expression patterns in grain of high and low asparagine wheat genotypes in response to sulphur supply**. *BMC Genom* 2019, **20**:628.
66. Emebiri LC: **Genetic variation and possible SNP markers for breeding wheat with low-grain asparagine, the major precursor for acrylamide formation in heat-processed products**. *J Sci Food Agric* 2014, **94**:1422-1429.
67. Rapp M, Schwadorf K, Leiser WL, Würschum T, Longin CFH: **Assessing the variation and genetic architecture of asparagine content in wheat: what can plant breeding contribute to a reduction in the acrylamide precursor?** *Theor Appl Genet* 2018, **131**:2427-2437.
68. •• Raffan S, Sparks C, Huttly A, Hyde L, Martignago D, Mead A, Hanley SJ, Wilkinson PA, Barker G, Edwards KJ, Curtis TY, Usher S, Kosik O, Halford NG: **Wheat with greatly reduced accumulation of free asparagine in the grain, produced by CRISPR/Cas9 editing of asparagine synthetase gene *TaASN2***. *Plant Biotech J* 2021, **19**:1602-1613.

This reference describes the use of CRISPR/Cas9 to produce wheat with very low concentrations of free asparagine in the grain, both per se and as a proportion of the free amino acid pool, with effects also on free glutamine, glutamate and aspartate.

69. •• Alarcón-Reverte R, Xie Y, Stromberger J, Cotter JD, Mason RE, Pearce S: **Induced mutations in *ASPARAGINE SYNTHETASE-A2* reduce free asparagine concentration in the wheat grain**. *Crop Sci* 2022, https://doi.org/10.1002/csc2.20760.

This reference describes the results of field trials of durum and bread wheat carrying ethyl methanesulphonate-induced mutations in the *TaASN-A2* gene, with 9–34% reductions in free asparagine concentration in the grain of the mutant lines.

70. •• Luo L, Qin R, Liu T, Yu M, Yang T, Xu G: ***OsASN1* plays a critical role in asparagine-dependent rice development**. *Int J Mol Sci* 2019, **20**:130.

This reference described rice lines lacking a functional *OsASN1* gene. In contrast to *TaASN2* knockouts of wheat [68,69], the plants showed effects on height, root length, and tiller number.

71. Halford NG: **Legislation governing genetically modified (GM) and genome edited crops in Europe: The need for change**. *J Sci Food Agric* 2019, **99**:8-12.
72. Paul V, Ezekiel R, Pandey R: **Sprout suppression on potato: need to look beyond CIPC for more effective and safer alternatives**. *J Food Sci Technol* 2016, **53**:1-18.
73. European Commission: **Directive 2009/128/EC of the European Parliament and of the Council of 21 October 2009 Establishing a Framework for Community Action to Achieve the Sustainable use of Pesticides**. European Commission; 2009.