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GENOTOXICITY ASSESSMENT OF SYNTHETIC FOOD COLOURANTS USING THE ALLIUM CEPA ASSAY

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Abstract

Synthetic colourants are among the most widely used food additives, and their genotoxic potential is still debated. This review, which reports no new experimental data, summarises what has been published about the Allium cepa root-tip assay as a screen for these compounds. It covers the test design, the endpoints scored, the colourants most often examined, the regulatory background and the biological mechanisms proposed for chromosomal damage. Published work shows that several azo and triphenylmethane dyes reduce the mitotic index and raise aberration and micronucleus frequencies at high concentrations, although results differ between laboratories. Inconsistent exposure protocols, the absence of metabolic activation in plant cells and weak links to human exposure remain the main gaps.

1. INTRODUCTION

Colour is one of the first things a consumer judges about a food. Manufacturers add synthetic dyes to confectionery, soft drinks, dairy desserts, pharmaceutical syrups and snack coatings because the dyes are cheap, stable and far more intense than most natural pigments. Surveys of foods eaten by children in the United States showed that a single serving of some sweets can carry a considerable dye load [1]. Most of the permitted dyes belong to the azo class, with a smaller group of triphenylmethane, xanthene and indigoid compounds. Because they are consumed daily and often for decades, even a weak effect on DNA would matter for public health, and that is why their safety has been revisited repeatedly [2, 3, 4, 5].

Genotoxicity testing of additives has traditionally relied on bacterial reverse mutation assays and mammalian cell tests, which are laid out in international guidelines [6, 7, 8]. These systems are sensitive but costly, and many laboratories in developing regions lack the facilities to run them routinely. Plant bioassays offer a cheaper alternative. The common onion, *Allium cepa*, has large chromosomes, a low diploid number (2n = 16) and roots that grow within days in plain water, so cytogenetic damage can be read directly under a light microscope [9, 10]. The assay has been used for decades to screen effluents, pesticides and food additives, which is the reason it now appears so often in studies of colourants.

Reports on food dyes in this test have multiplied, yet the conclusions are not easy to compare. Some papers describe a clear dose-dependent fall in mitotic activity with abundant chromosome breaks, while others find only mild effects, or effects confined to the highest concentrations tested. Part of the confusion arises from differences in exposure time, dye purity, concentration range and scoring rules. This review brings together the published evidence on the use of the *Allium cepa* assay for synthetic food colourants. It does not present original experiments. The aim is to describe how the test works, what it has shown for the common dyes, how the findings relate to regulatory decisions and where the interpretation is weakest.

The chemistry of the dyes explains much of the biology. Azo colourants carry one or more nitrogen–nitrogen double bonds linking aromatic rings, and the sulphonate groups added during manufacture make them soluble and discourage absorption across the intestinal wall. Early bacterial work by Ames and colleagues established the principle that many carcinogens are detectable as mutagens once they are metabolically activated, and that principle shaped how azo compounds have been screened ever since [11]. Plant assays came into use from a different tradition, that of environmental monitoring, and the two traditions only recently began to converge on food additives. Reading the plant literature therefore means keeping both histories in mind, because the questions asked and the doses applied differ.

Several clarifications about scope are needed. Only colourants permitted for use in foods and beverages are considered, with passing mention of textile dyes where the same chemistry is relevant. Natural pigments such as anthocyanins and carotenoids fall outside the discussion. Evidence from bacteria, cultured cells and rodents is used for context, because a plant result has little meaning unless it is set against what other systems report [12, 13, 14]. The article follows the sequence of the experimental workflow, beginning with the biology of the test and ending with a reading of its limits, so that a worker planning a study can see which choices influence the answer.

2. THE ALLIUM CEPA TEST SYSTEM

The classical protocol grows bulbs in water until the roots reach about two to three centimetres, then transfers them to the test solution for a fixed period, commonly 24 to 72 hours. Root tips are fixed, hydrolysed in hydrochloric acid, stained with acetocarmine or orcein and squashed on a slide. Fiskesjö proposed the root-growth inhibition measure and the cytological scoring that still form the core of most studies [9]. A negative control in tap or distilled water and a positive control are always included. Methyl methanesulfonate, maleic hydrazide and sodium azide have all served as reference mutagens, and the response of the positive control is how a laboratory proves its test is working [15].

Several endpoints are read from the same slides. The mitotic index, the proportion of dividing cells among all cells counted, gives a measure of cytotoxicity, since a toxic compound slows or stops the cycle. Chromosomal aberrations are scored at anaphase and telophase, where breaks, bridges, lagging chromosomes and fragments can be seen without ambiguity [15]. Micronuclei are counted in interphase cells and indicate either chromosome breakage or loss of whole chromosomes at division [16]. Nuclear abnormalities such as lobed nuclei, bud-like protrusions and binucleate cells are recorded by some groups as additional signs of stress, though their meaning is less settled.

Counting a minimum of several thousand cells per treatment is generally advised to give stable estimates [17].

Why does this plant behave as a reliable indicator? Grant's review of plant cytogenetics noted that the chromosome aberrations found in plants after exposure to chemicals generally agree with those in mammalian cells, which gave early support to the use of root tips as a monitoring tool [18]. Later comparisons by Leme and Marin-Morales reported good agreement between onion results and those from other eukaryotic assays for many classes of pollutant, while pointing out that agreement is not perfect [10]. Colchicine, whose effect on root mitosis in *Allium* was described as early as 1938, remains a textbook example of a spindle poison that produces the c-mitosis pattern in this system [19].

Standardisation has lagged behind popularity. Bonciu and colleagues reviewed hundreds of papers and found wide variation in bulb size and origin, germination medium, treatment duration, fixation, staining, the number of cells scored and even the definition of an aberration [17]. Some authors treat whole bulbs, others use seedlings or isolated roots. Cultivar matters too, because a bulb grown for the market may carry its own background level of cytological abnormality. The authors recommended a harmonised procedure and clear reporting of every step. Until such a protocol is adopted widely, two studies on the same dye can reach different conclusions simply because they asked slightly different questions of the root.

Root length offers a macroscopic endpoint that complements the slide-based measures. Bundles of roots are measured after exposure, and the concentration that halves elongation relative to the water control is used to summarise toxicity, as proposed in the original protocol [9]. The value is cheap to obtain and does not depend on a trained cytologist, which makes it attractive for ranking several dyes or effluent dilutions before anything is fixed and stained. It does not always move in step with the cytological scores, however. A dye may slow elongation through effects on cell expansion or water uptake with little change in mitotic activity, so growth inhibition and chromosome damage should be reported separately and interpreted with caution [9, 10]. Recording the exposure period and the temperature of the room alongside the measurements also helps, because root elongation in water itself varies from one batch of bulbs to another [9].

Treatment design shapes what the assay can detect. A continuous exposure of 24 to 48 hours captures effects on cells that pass through the cycle during treatment, while a short exposure followed by a recovery period in water allows scoring of damage that persists after the stress has been removed. Recovery experiments are particularly useful for separating transient mitodepression from lasting chromosomal injury. Concentration series should span at least three or four levels, ideally chosen from a preliminary root-growth test in which the concentration that halves root elongation is identified [9]. Lower levels then show whether any genotoxic signal appears before frank toxicity, a requirement also written into mammalian cell guidelines to avoid false positives from dying cells [7].

A practical point deserves emphasis. Root cells have no cytochrome P450 system comparable with the mammalian liver, so a compound that needs metabolic activation to become reactive may pass unnoticed. Plant tissues do possess peroxidases and other enzymes capable of transforming xenobiotics, and some activation clearly takes place, but its relevance for compounds that humans metabolise through gut bacteria and hepatic enzymes is uncertain [10]. Azo dyes are a case in point. Their most reactive products, aromatic amines, are generated largely by azoreductases of the intestinal flora [20]. A negative result in the onion root therefore says less about an azo dye than a positive one.

3. FOOD COLOURANTS AND REGULATION

The dyes most often tested in the onion are tartrazine (E102), sunset yellow FCF (E110), allura red AC (E129), carmoisine (E122), ponceau 4R (E124), amaranth, erythrosine and brilliant blue FCF. The first five are monoazo compounds containing one or more sulphonate groups, which make them highly

water soluble and poorly absorbed from the gut. Erythrosine is a xanthene dye with four iodine atoms, and brilliant blue is a triphenylmethane compound. Water solubility has a practical consequence for the root assay, because the dyes can be dissolved directly in the treatment medium at the concentrations of interest, whereas poorly soluble substances need solvents that may themselves disturb division.

Regulation is based on acceptable daily intake values set after toxicological review. In Europe all permitted colours were re-evaluated by the European Food Safety Authority in the late 2000s, and the opinion on tartrazine concluded that the available genotoxicity data did not raise concern at permitted levels, while recommending that specifications and exposure estimates be refined [3]. Work on single dyes such as quinoline yellow, and on panels of dyes in the fruit-fly somatic mutation and recombination test, has given results that depend on the dye and the concentration used [4, 5]. Use is governed by the framework regulation on food additives, which lists approved colours and maximum levels per food category [21]. International trade relies on the Codex general standard for food additives [22], and India applies its own food additive regulations through the national food safety authority [23].

Public attention was sharpened in 2007 when a randomised, double-blind trial in British children reported that mixtures of certain colours and a preservative increased hyperactive behaviour in the general population [24]. The study did not concern genotoxicity, yet it triggered label warnings in the European Union and prompted regulators to look again at toxicological databases. A systematic review of the older literature by Kobylewski and Jacobson went further and argued that several dyes had incomplete or poorly designed studies, with some positive genotoxicity findings that deserved follow-up [2]. This scepticism about the adequacy of the evidence is one reason independent laboratories continue to test colourants in inexpensive assays such as the onion.

Commercial dye preparations are never perfectly pure. Specifications allow a stated percentage of subsidiary colouring matters, uncombined intermediates, unsulphonated primary aromatic amines, sodium chloride and sodium sulphate, and the assessments by European regulators led to tighter limits for some of these impurities [3]. For research on genotoxicity this matters in two ways. A positive result may be driven by a trace impurity and not by the principal chromophore, and two suppliers can sell products of different composition under the same name. Reporting the supplier, batch and analytical purity is simple, yet many plant studies leave it out, which makes replication difficult and weakens any comparison between laboratories.

Exposure is the other side of risk. Dyes are not eaten as pure substances but in foods, usually at low milligram amounts per serving, and a child's intake relative to body weight can be several times that of an adult. The study of common children's foods mentioned earlier showed that a few products carry tens of milligrams of dye per serving [1]. By contrast, the concentrations used in plant studies often run to hundreds or thousands of milligrams per litre. Such concentrations are relevant to wastewater from dyeing and food processing plants, but they are far above the likely concentration in a person's tissues. Any reading of onion results for dietary risk has to acknowledge this gap openly.

4. REPORTED GENOTOXIC FINDINGS

The representative findings gathered in table 1 come from the published literature and are grouped by the system in which they were obtained. Taken at face value the picture is mixed. Cytogenetic damage by azo dyes has been described in plant cells and in cultured mammalian cells at high concentrations [14, 25, 26], whereas bacterial mutation assays and in vivo micronucleus tests in rodents are usually negative or equivocal for the same compounds [27, 28, 29]. This pattern is summarised numerically in fig. 1, which counts entries by test system. It should be read as a map of what has been studied, not as a verdict on any individual dye.

In onion roots, the common outcome of exposure to azo dyes is a drop in the mitotic index, followed by chromosome stickiness,

bridges, laggards, fragments and micronuclei as concentration or time increases. Effluents from silk and textile dyeing, which contain azo dyes together with salts and auxiliary chemicals, reduced mitotic activity and produced a spectrum of aberrations in treated roots [25]. Experiments in which seeds were exposed to industrial effluent contaminated with azo dyes gave a similar pattern and linked the damage to the dye content [26]. These studies concerned industrial colourants, so their value for food dyes lies mainly in showing that the chemical class can disturb root mitosis.

Table 1: Representative published genotoxicity findings for synthetic food and related colourants

Colourant or sample	Reported finding	Ref.
Silk dyeing effluent azo dyes	Lower mitotic index and varied aberrations in onion root cells	[25]
Azo-dye-contaminated effluent seed exposure	Chromosome aberrations linked to dye content in onion tests	[26]
Food preservatives benzoate, sorbate	Concentration-dependent fall in mitosis and rise in aberrations	[30]
Tartrazine, amaranth, erythrosine	Altered chromosomes in human lymphocytes; DNA interaction in vitro	[14]
Japanese food additive screen dyes	Chromosome aberrations in fibroblasts, mostly at high concentration	[31]
Sulphonated azo dyes Salmonella	Mutagenicity generally needed reductive cleavage of azo bond	[27]
39 food additives mouse comet assay	DNA damage in gut after some dyes at high oral doses	[12]
Red dyes in mice oral dosing	DNA damage reported in stomach and colon	[13]
Amaranth, sunset yellow, tartrazine	No genotoxic effect in mouse gut micronucleus assay	[28]
Allura red AC	No genotoxicity in a set of in vivo studies	[29]

Dose-response behaviour in the onion deserves a closer look. In most reports the mitotic index declines as concentration rises, then reaches a plateau, while the frequency of aberrant cells peaks at an intermediate concentration and may fall at the highest one, where few cells divide at all. A falling aberration rate at the top of the range is easy to misread as a protective effect. It is usually the arithmetic consequence of cytotoxicity, since the pool of dividing cells shrinks. Authors who express aberrations per dividing cell, or who present the mitotic index alongside, make this clear. The same shape appears in textile effluent studies [25, 26], and it is worth expecting for any colourant that is toxic enough to reach the plateau.

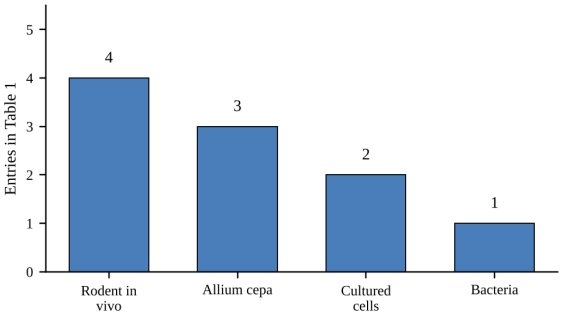


Fig. 1: Number of entries in table 1 by test system

A note on consistency helps here. When the same dye is tested at similar concentrations in different laboratories, the mitotic index results usually agree in direction, but the aberration counts differ several-fold. That kind of scatter is typical for subjective microscopic scoring, and it was one of the motives behind calls for standard operating procedures [17]. Between-laboratory variation is also a reminder that a single positive paper on a given dye should be treated as a signal to repeat the work. Convergence across independent groups, each using blinded scoring and a clear positive control, would carry far more weight than any one striking result.

For permitted food dyes the plant evidence is thinner and the direction of effect depends on the compound. Tartrazine, amaranth and erythrosine were examined in cultured human lymphocytes, where all three altered chromosome behaviour and showed some ability to interact with DNA in vitro [14]. Work on food preservatives in *Allium cepa* provided a methodological template, reporting concentration-dependent falls in the mitotic index and increases in aberrations for sodium benzoate, potassium sorbate and related additives [30]. Investigators studying colourants have followed that design and

generally report cytotoxic and clastogenic effects at the upper end of the dose range, with weak or absent effects at the lower end.

Mammalian data complicate the picture. The comet assay in eight mouse organs after oral dosing of 39 food additives detected DNA damage in the gastrointestinal tract after some dyes, including several red and yellow compounds, although the doses were far above normal intake [12]. Other mouse studies reported DNA damage in the stomach and colon after oral administration of red dyes such as allura red and amaranth [13]. Yet a well-controlled gut micronucleus assay in mice found no genotoxic effect of amaranth, sunset yellow and tartrazine or of their metabolites [28], and an extensive in vivo programme on allura red concluded the dye was not genotoxic [29]. Differences in dose, route and endpoint appear to explain much of this divergence.

Older screening work should not be forgotten. A Japanese programme tested hundreds of food additives in bacterial and chromosome aberration assays and reported positive chromosome findings for a number of synthetic dyes in cultured fibroblasts, usually at high concentrations and often without a clear dose response [31]. Studies of sulphonated azo dyes in *Salmonella* showed that mutagenicity generally required reductive cleavage of the azo bond, and that the native sulphonated molecules were largely inactive [27]. These observations fit the view that parent food dyes are poorly reactive and that risk, if any, comes from reduction products. A plant test that lacks gut bacteria cannot reproduce that step, which explains many of the apparent disagreements.

Biochemical studies add a different dimension. Young rats given tartrazine or carmoisine by mouth showed raised liver and kidney function markers and altered indices of oxidative stress in one report [32], which supports the idea that these dyes can burden tissues at sufficiently high intakes. Such results do not demonstrate DNA damage directly, yet oxidative stress is a recognised route to strand breaks and base oxidation. The point is not that every dye is harmful, but that different laboratories, using different systems, repeatedly find signs of cellular stress at supraphysiological doses. The plant assay is one more instrument in that chorus, and it should be weighed with the others.

5. PROPOSED MECHANISMS

Mechanistic explanations offered for dye-induced damage in the onion fall into a few groups. Direct interaction with DNA has been considered for planar aromatic molecules that can intercalate between bases, and binding to calf thymus DNA was demonstrated in vitro for some food dyes [14]. Indirect action through the mitotic apparatus is also proposed. Compounds that interfere with spindle formation produce lagging chromosomes, c-metaphases and polyploid cells, which is the signature of an aneugenic rather than a clastogenic agent, as in the classical action of colchicine [19]. Distinguishing between these routes requires careful scoring of the type of aberration, which is why reporting the full spectrum matters.

Micronuclei form by more than one process. Fragments from broken chromosomes give rise to micronuclei lacking a centromere, while lagging whole chromosomes give centromere-positive ones. In onion root cells exposed to the herbicide trifluralin, a well-known aneugen, micronuclei arose chiefly from polyploidised cells, and the authors traced a pathway from spindle disturbance to nuclear buds and then to micronuclei [33]. If food dyes act in the same way, a rise in micronuclei would reflect disturbed segregation rather than DNA breakage. Few colourant studies have attempted to apply the staining or labelling methods that would settle the question, leaving the mechanism largely inferred from morphology.

Oxidative stress is the third candidate. Reactive oxygen species are produced in all aerobic cells and are kept in check by antioxidant enzymes, but excess production damages lipids, proteins and nucleic acids [34]. Dyes might raise that burden by redox cycling, by depleting glutathione or by disturbing mitochondrial function. Measurements of lipid peroxidation, catalase and superoxide dismutase activity in treated onion roots would help here, and the rodent

biochemical data point in the same direction [32]. A causal chain from dye to oxidant to DNA lesion is plausible, but in most papers it is assumed rather than tested, since the cytological slides and the enzyme assays are rarely reported together.

Cell cycle control also deserves mention. Checkpoints delay progress through the cycle when DNA is damaged or the spindle is defective, allowing repair before division [35]. A lowered mitotic index may thus signal activation of a checkpoint rather than simple cell death, in which case cells accumulate in G2 or interphase. If the damage is overwhelming, checkpoint failure lets abnormal cells enter mitosis and produce the aberrations seen at anaphase. This view helps to explain why the mitotic index usually falls first, at low concentrations, and why aberrations appear mainly at higher ones. It also suggests that a sample with a depressed mitotic index and few aberrations should be called cytotoxic, not genotoxic.

Gut bacteria turn the discussion from the plant back to the consumer. Intestinal anaerobes reduce the azo linkage of dyes in a reaction that has been demonstrated with human faecal flora and with rodent caecal contents, releasing aromatic amines [20]. For sulphonated food dyes the amines formed are usually sulphanilic or naphthionic acid derivatives, which carry the same charged group and are mostly excreted unchanged, whereas amines produced from non-sulphonated or industrial dyes can be absorbed and activated in the liver [36]. Whether this difference accounts for the generally reassuring in vivo data on permitted dyes remains an open question, but it is the most coherent explanation available at present.

Biotransformation in the intact organism is the last element. In humans and rodents, azo dyes are split by bacterial azoreductases in the lower gut into aromatic amines, some of which, such as certain benzidine derivatives, are established carcinogens, whereas the amines released from the sulphonated food dyes are themselves sulphonated and generally poorly absorbed [20, 36]. This is the central reason why regulators place weight on in vivo data and why an unmetabolised dye in an onion root is an imperfect model of dietary exposure. It does not make the plant result meaningless, as it still detects intrinsic reactivity, but it does set a ceiling on what can be inferred.

Polyploidy and endoreduplication are sometimes seen after dye exposure and might be interpreted as a stress response. Plants cope with injury by shifting some cells into extra rounds of DNA replication without division, which makes the nuclei larger and the cells less able to complete a normal mitosis. Such events are visible as enlarged nuclei with condensed prophase figures, and they can be mistaken for aberrations when the observer is not trained to recognise them. Counting rules that separate true structural damage from ploidy changes would reduce subjectivity, and photographing representative cells for each category gives readers a chance to judge the scoring for themselves.

6. INTERPRETATION AND LIMITATIONS

How should a positive onion result be read? A reasonable view is that it signals hazard, not risk. It shows that a compound at a given concentration can disturb mitosis in a plant root, and it may justify further testing in a regulatory battery. It does not predict carcinogenicity in people. Guidance for pharmaceuticals recommends that a test for gene mutation in bacteria be combined with a cytogenetic test in mammalian cells and an in vivo assay [8], and the same logic should apply to additives. In that framework the onion belongs at the screening end, where it can rank substances or flag effluents, but cannot carry decisions about acceptable intake.

Concentration choice is a recurring weakness. Many papers use dye concentrations expressed in milligrams per litre without relating them to dietary levels or to effluent measurements, and some do not report purity or the presence of subsidiary colours and salts, which can make up a substantial share of commercial preparations. Contaminants may contribute to toxicity as much as the main dye. Reporting the specification of the material, the vehicle, the pH of the solution

and the stability of the dye under the test conditions would make results more transferable. Light-induced degradation is another variable, since some dyes change in the open dishes used for bulb exposure.

Statistical treatment is often thin. Counting a few hundred cells per slide, pooling slides from different bulbs and applying a simple chi-square test may overstate precision, because the experimental unit is the bulb or root, not the cell. Better practice uses several independent bulbs per treatment, scoring blinded to treatment, and a model that accounts for variation between roots. The recommended comparison is to a concurrent negative control with a historical range kept by the laboratory, and a positive control with a confirmed response [15, 17]. Without such controls an apparent effect might be nothing more than the normal variation of a biological system that is easy to stress.

For the laboratory planning a new study, a few practical rules follow from the literature. Specify the dye, supplier and purity. Choose concentrations from a range-finding test and include at least one level close to realistic exposure. Run negative and positive controls concurrently, and score at least 1,000 cells for each of several independent bulbs, preferably with the slides coded [15, 17]. Report mitotic index, the type of aberrations and micronuclei separately. Where a positive effect is found, confirm it in a second assay with different biology, for example the comet assay or the in vitro micronucleus test [37, 38]. These steps cost little, and they would remove most of the ambiguity found in the current evidence.

The biggest gap remains the link to human exposure. Few studies measured uptake of dye into root cells, and none could show that the intracellular concentration approached anything a consumer would experience. Future work could combine the onion assay with the comet assay, which detects DNA strand breaks in individual nuclei [37], and with in vitro micronucleus tests in mammalian cells under validated conditions [7, 38, 39]. Adding an azoreduction step, for example by pre-incubating the dye with a bacterial or liver fraction, would make the plant model more informative for azo compounds. Harmonised protocols and open data sharing would make it possible to pool studies in a meta-analysis.

Regulatory use of such data is conditional. The standard bacterial and mammalian cell guidelines specify strain sets, metabolic activation systems, treatment schedules and acceptance criteria that plant bioassays do not yet have [6, 7]. For this reason, an onion study cannot replace those tests in a dossier. What it can do is provide supporting evidence of cytogenetic activity, rank closely related compounds, and monitor effluents from food and textile factories where the hazard comes from mixtures. In low-resource settings it can also train students in cytogenetics and give local authorities a first indication of pollution. Setting expectations at this level avoids both overselling and unfair dismissal of a useful tool.

7. CONCLUSION

The onion root assay is a simple, inexpensive and informative first screen for the cytotoxic and clastogenic potential of colourants. The published work shows that azo dyes and effluents containing them can reduce mitotic activity and raise chromosomal aberrations and micronuclei in root cells, usually at high concentrations, while permitted food dyes give variable results in the plant and mostly negative or equivocal results in well-controlled mammalian studies [12, 25, 28, 29]. Mechanisms proposed include direct DNA binding, spindle disturbance, oxidative stress and checkpoint failure [14, 33, 34, 35]. None has been proved for food dyes in this plant system.

Reading these findings for human safety needs caution. Plant roots lack gut bacterial reduction, exposure levels differ by orders of magnitude from dietary intake, and protocols are not harmonised [1, 2, 17]. Future work should report dye purity, use biologically sensible concentrations, score cells blind with proper controls, and pair the onion with the comet assay or a mammalian micronucleus test [7, 37, 38]. Regulatory conclusions should continue to rest on the full battery of tests and on exposure assessment [3, 4, 5, 8], with the onion serving as a screening and monitoring tool, particularly valuable for dye-bearing wastewater [10, 26].

8. ABBREVIATIONS

ADI-Acceptable daily intake, DNA-Deoxyribonucleic acid, EFSA-European Food Safety Authority, FSSAI-Food Safety and Standards Authority of India, ICH-International Council for Harmonisation, MI-Mitotic index, OECD-Organisation for Economic Co-operation and Development, ROS-Reactive oxygen species

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Nil

10. AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

11. CONFLICT OF INTERESTS

Declared none

12. REFERENCES

1. Stevens LJ, Burgess JR, Stochelski MA, Kuczek T. Amounts of artificial food dyes and added sugars in foods and sweets commonly consumed by children. Clin Pediatr 2015;54:309–321.
2. Kobylewski S, Jacobson MF. Toxicology of food dyes. Int J Occup Environ Health 2012;18:220–246.
3. EFSA Panel on Food Additives and Nutrient Sources added to Food. Scientific opinion on the re-evaluation of Tartrazine (E 102). EFSA J 2009;7:1331.
4. Macioszek VK, Kononowicz AK. The evaluation of the genotoxicity of two commonly used food colors: Quinoline Yellow (E 104) and Brilliant Black BN (E 151). Cell Mol Biol Lett 2004;9:107–22.
5. Sarıkaya R, Selvi M, Erkoç F. Evaluation of potential genotoxicity of five food dyes using the somatic mutation and recombination test. Chemosphere 2012;88:974–9.
6. Organisation for Economic Co-operation and Development. Test No. 471: Bacterial reverse mutation test. Paris: OECD; 1997.
7. Organisation for Economic Co-operation and Development. Test No. 487: In vitro mammalian cell micronucleus test. Paris: OECD; 2016.
8. ICH Harmonised Tripartite Guideline. Guidance on genotoxicity testing and data interpretation for pharmaceuticals intended for human use S2(R1). Geneva: ICH; 2011.
9. Fiskesjö G. The Allium test as a standard in environmental monitoring. Hereditas 1985;102:99–112.
10. Leme DM, Marin-Morales MA. Allium cepa test in environmental monitoring: a review on its application. Mutat Res 2009;682:71–81.
11. Ames BN, McCann J, Yamasaki E. Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mutat Res 1975;31:347–364.
12. Sasaki YF, Kawaguchi S, Kamaya A, Ohshita M, Kabasawa K, Iwama K, *et al.* The comet assay with 8 mouse organs: results with 39 currently used food additives. Mutat Res 2002;519:103–119.
13. Tsuda S, Murakami M, Matsusaka N, Kano K, Taniguchi K, Sasaki YF. DNA damage induced by red food dyes orally administered to pregnant and male mice. Toxicol Sci 2001;61:92–99.
14. Mpountoukas P, Pantazaki A, Kostareli E, Christodoulou P, Kareli D, Poliliou S, *et al.* Cytogenetic evaluation and DNA interaction studies of the food colorants amaranth, erythrosine and tartrazine. Food Chem Toxicol 2010;48:2934–2944.
15. Rank J, Nielsen MH. Allium cepa anaphase-telophase root tip chromosome aberration assay on N-methyl-N-nitrosourea, maleic hydrazide, sodium azide, and ethyl methanesulfonate. Mutat Res 1994;312:17–24.
16. Ma TH, Xu Z, Xu C, McConnell H, Rabago EV, Arreola GA, *et al.* The improved Allium/Vicia root tip micronucleus assay for clastogenicity of environmental pollutants. Mutat Res 1995;334:185–195.
17. Bonciu E, Firbas P, Fontanetti CS, Wusheng J, Karaismailoğlu MC, Liu D, *et al.* An evaluation for the standardization of the Allium cepa test as cytotoxicity and genotoxicity assay. Caryologia 2018;71:191–209.
18. Grant WF. Chromosome aberrations in plants as a monitoring system. Environ Health Perspect 1978;27:37–43.
19. Levan A. The effect of colchicine on root mitoses in Allium. Hereditas 1938;24:471–486.
20. Chung KT, Stevens SE Jr, Cerniglia CE. The reduction of azo dyes by the intestinal microflora. Crit Rev Microbiol 1992;18:175–190.
21. European Parliament and Council. Regulation (EC) No 1333/2008 on food additives. Off J

Eur Union 2008;L354:16–33.

22. Codex Alimentarius Commission. General standard for food additives CODEX STAN 192-1995. Rome: FAO/WHO; 1995.
23. Food Safety and Standards Authority of India. Food Safety and Standards (Food Products Standards and Food Additives) Regulations, 2011. New Delhi: FSSAI; 2011.
24. McCann D, Barrett A, Cooper A, Crumpler D, Dalen L, Grimshaw K, *et al.* Food additives and hyperactive behaviour in 3-year-old and 8/9-year-old children in the community: a randomised, double-blinded, placebo-controlled trial. Lancet 2007;370:1560–1567.
25. Sudhakar R, Ninge Gowda KN, Venu G. Mitotic abnormalities induced by silk dyeing industry effluents in the cells of Allium cepa. Cytologia 2001;66:235–239.
26. Caritá R, Marin-Morales MA. Induction of chromosome aberrations in the Allium cepa test system caused by the exposure of seeds to industrial effluents contaminated with azo dyes. Chemosphere 2008;72:722–725.
27. Brown JP, Dietrich PS. Mutagenicity of selected sulfonated azo dyes in the Salmonella/microsome assay. Mutat Res 1983;116:305–315.
28. Poul M, Jarry G, Elhkim MO, Poul JM. Lack of genotoxic effect of food dyes amaranth, sunset yellow and tartrazine and their metabolites in the gut micronucleus assay in mice. Food Chem Toxicol 2009;47:443–448.
29. Bastaki M, Farrell T, Bhusari S, Pant K, Kulkarni R. Lack of genotoxicity in vivo for food color additive Allura Red AC. Food Chem Toxicol 2017;105:308–314.
30. Türkoğlu S. Genotoxicity of five food preservatives tested on root tips of Allium cepa L. Mutat Res 2007;626:4–14.
31. Ishidate M Jr, Sofuni T, Yoshikawa K, Hayashi M, Nohmi T, Sawada M, *et al.* Primary mutagenicity screening of food additives currently used in Japan. Food Chem Toxicol 1984;22:623–636.
32. Amin KS, Hameid HA, Elsttar AHA. Effect of food azo dyes tartrazine and carmoisine on biochemical parameters related to renal, hepatic function and oxidative stress biomarkers in young male rats. Food Chem Toxicol 2010;48:2994–2999.
33. Fernandes TCC, Mazzeo DEC, Marin-Morales MA. Mechanism of micronuclei formation in polyploidizated cells of Allium cepa exposed to trifluralin herbicide. Pestic Biochem Physiol 2007;88:252–259.
34. Mittler R. ROS are good. Trends Plant Sci 2017;22:11–19.
35. Hartwell LH, Weinert TA. Checkpoints: controls that ensure the order of cell cycle events. Science 1989;246:629–634.
36. Chung KT. Azo dyes and human health: a review. J Environ Sci Health C 2016;34:233–261.
37. Collins AR. The comet assay for DNA damage and repair: principles, applications, and limitations. Mol Biotechnol 2004;26:249–261.
38. Fenech M. The in vitro micronucleus technique. Mutat Res 2000;455:81–95.
39. Kirsch-Volders M, Sofuni T, Aardema M, Albertini S, Eastmond D, Fenech M, *et al.* Report from the in vitro micronucleus assay working group. Mutat Res 2003;540:153–163.