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Ethidium brouhaha: exorcising the EtBr demon from wimpy researchers

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Abstract

Ethidium bromide is a celebrity stain, and a very tainted one, in molecular biology. Its stigmatisation as a toxic, carcinogenic and mutagenic chemical has stirred an uncalled-for but what seems like a calculated hysteria among researchers. This has merely actuated what can be described as a necessary advertising war. The so-called safer alternatives are not overwhelmingly superior or entirely safe. For example, SYBR Green is, by evidence, more mutagenic. They may be regarded as optional, but at a higher financial cost. If anyone feels safer with extravagance, then the optional stains are an obvious choice. EtBr had been the principal drug for the mass treatment of cattle trypanosomiasis since the early 1950s, and no cancer has been reported among cattle. There are researchers who has handled the chemical throughout their career for decades, and none had made any complaint whatsoever. There is no medical record of molecular biologists taking cancer therapy because of EtBr. Milk producers are using it in large quantities. To the further extreme, people have actually drunk it without any apparent adverse effect. This is a lesson to learn that this is a case of bad meme and people have put an undue stain to this useful stain.

Key words: Ethidium bromide; carcinogen; toxic; safety.

What EtBr is

Ethidium bromide (EtBr) is the most popular DNA intercalating agent, and was once the most widely nucleic acid stain. It was developed in the early 1950s as a drug for treating trypanosomiasis, a parasitic disease characterised by sleeping sickness, in cattle. It was by pure serendipitous invention that it was used in gel electrophoresis as DNA marker. Its ability to get inserted between the base pairs, and its bright orange-red luminescence made it an ideal stain. Its application has extended in milk industry where they use it for large-scale testing of milk quality. [For an excellent– in my egoistic judgement – but rather boring review, see the preceding article,[1] you will not regret it.]

EtBr is so indispensable that every laboratory that has even the vaguest connection with DNA work – RNA and proteins not mentioning – will

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Figure 1. Different brands of EtBr, the same chemical of disrepute but has saved the lives of cattle and sheep from trypanosomiasis.

be proud to display it. It is the most widely used drug for cattle trypanosomiasis (Fig. 1), and most widely used nucleic acid stain that it is the most studied compound among the phenanthridines. There have been serious health concerns for its toxicity and mutagenicity in cultured cells. It is precisely for these reasons that it has earned a cohort of ill-reputed monikers such as toxic, genotoxic, mutagen, carcinogen, hazardous, and teratogen. This has created fear and furore in molecular laboratories all over the world, and coincidentally opens the floodgate for rival products to toot their own horn.

This article assumes to highlight the irrationality of the terror emanating from the off-putting hue and cry against EtBr as a menacing chemical, which is not at all justified by scientific evidences. In fact, most, if not all, the warnings are wildly exaggerated claims.

THE ROOT OF EVIL

According to the MIT (Massachusetts Institute of Technology) Green Chemistry Case Study, the drawbacks of using EtBr include:[2]

- ⊕ it can be absorbed through the skin, irritating the eyes, mouth, and upper respiratory tract;
- ⊕ because of its tendency to intercalate in DNA bands, ethidium bromide is a powerful mutagen;
- ⊕ if handled indiscriminately in the lab, ethidium bromide can easily contaminate a large work area. When lab spaces are prepared for a move or for renovation, the space must be decontaminated of chemical, biological and radiological hazards. Because individual laboratories bear most, if not all, of the cost of decontaminating a lab, widespread ethidium bromide contamination may unnecessarily increase either the time or cost of lab preparation for moves or renovations; and
- ⊕ techniques for managing ethidium bromide waste are expensive – from a materials perspective, labor perspective, or both – or they beget more waste.

EtBr is unequivocally shown to be genotoxic, a frame-shift mutagen and teratogen. But the crux of the story to appreciate is that experiments showing these dreadful properties are not in whole animals. But they are examined by *in vitro* tests on various cultured cell lines and embryo systems. It is the experimental results from these cells that the all the horrifying accusations of EtBr hazards erupted. These cultured cells indicated that EtBr can cause frame-shift mutations, chromosomal recombination, impeded cell cycle and various developmental anomalies.

EtBr can cause severe malformations in a Frog Embryo Teratogenesis Assay: Xenopus (FETAX). Three-week-old South African frog *(Xenpous laevis)* larvae exposed to near toxic EtBr

concentrations developed gross malformations of all major organ systems, including spinal curvature, anencephaly, microcephaly, and microphthalmia. The LC_{50} for EB in this system was 0.05 mg/ml while the EC_{50} for malformation was 0.035 mg/ml.[3] Nass (1972) indicated that the growth of mouse fibroblasts and baby hamster kidney cells was completely inhibited by concentrations of 0.1-5 g/ml.[4]

On the imaginative side, I wonder if such a mutagenic drug had been applied for decades (Fig. 2), why mutant cows have not been roaming the continent of Africa. To reiterate, EtBr was originally developed and had served as a drug of choice for the treatment of cattle trypanosomiasis for several decades. Although the obvious lack of existence of Supercow (as we have fictional Superman), or X-cow (for counterpart of the X-Men), or a cow of any sort remotely possessing superpower [in fact I fail to conceive any fictional superhero made of cow, but there is a video game named "Super Cow"], may not be a convincing evidence of absence of mutant cows, but it suggests that there really is not a shred of evidence that EtBr can cause detrimental effects on large animals. There is not even a single report of malformed cows due to EtBr.

Figure 2. No cows were killed in the making of this drug, EtB, other than pricking with a syringe needle; in fact, it saved them.

The obvious paradox was tersely stated by Rosie Redfield:[5]

> The recommended dose for cattle is 1 mg/kg body weight (up to 50 mg/kg has been used in mice). Compare this with the 0.25-1 microgram/ml used in molecular biology. A 50 kg researcher would need to drink 50 liters of gel-staining solution [EtBr] to get even the non-toxic dose used in cattle.

We should learn to compromise with our complex physiological construction before we succumb to any medical threat. We, vertebrates of all persuasions – atheists and religious fundamental snobs including – are inherently blessed with an organ called the liver. This is mightily effective in destroying or eliminating toxic substances that we ingest. It performs this detoxifying activity routinely to keep us normally alive. Take alcoholic liver cirrhosis for an example. Alcohol actively destroys liver cells (cirrhosis), but upon abstinence, liver has the ability to regenerate its normal structure and function. The implication is of biblical proportion, because if the liver is not such an effective repairing device, most people would die of alcohol. In fact, our species could have been wiped off, as our forefathers started drinking some 12,000 years ago.[6]

...AND IS NOT

To recap the previous statement, the whole lot of empirical evidences on the hazards of EtBr lies entirely on *in vitro* tests using isolated cultured cells, but not substantiated on any metazoan like us. No positive or negative epidemiological studies or case reports associating EB ei-

ther positively or negatively with a cancer risk in humans are to be found in the medical literature (Fig. 3).

Figure 3. Some informed campaigns are just plain derogatory and lack empirical evidence such as this. Mercury, lead, and formalin are known highly toxic and poisonous chemicals. The same is not true for EtBr.

According to the Material Safety Data Sheet of Fisher Scientific:[7]

> Ethidium Bromide:
> Toxicity (inhalation, rat): 0.0118 - 0.1340 mg/L/6H. (oral,rat): 1503 mg/kg.
> Carcinogenicity:
> CAS# 7732-18-5: Not listed by ACGIH, IARC, NTP, or CA Prop 65.
> CAS# 1239-45-8: Not listed by ACGIH, IARC, NTP, or CA Prop 65.
> Epidemiology: No information found
> Teratogenicity: No information found
> Reproductive Effects: No information found
> Mutagenicity: Possible mutagenic effect in humans. The suspicion is based on proven damage to the genetic material in the somatic cells of man and animals and requires further clarification.
> Neurotoxicity: No information found
> Clean Air Act:
> This material does not contain any hazardous air pollutants.
> This material does not contain any Class 1 Ozone depletors.
> This material does not contain any Class 2 Ozone depletors.
> Clean Water Act:
> None of the chemicals in this product are listed as Hazardous Substances under the CWA.
> None of the chemicals in this product are listed as Priority Pollutants under the CWA.
> None of the chemicals in this product are listed as Toxic Pollutants under the CWA.
> OSHA:
> None of the chemicals in this product are considered highly hazardous by OSHA.

Look at the toxicity, just to make comparison, EtBr is much less toxic than aspirin (LD_{50} 200 mg/kg) and caffeine (LD_{50} 192 mg/kg), and consider how much people regularly take these medicinal compounds regularly.

The National Toxicology Program of the US Department of Health and Human Services documented that EtBr is genetoxic to the bacterium *Salmonella* but not in mice.[8]

There was a study of EtBr effect on human lymphocytes, in which it was shown that a concentration of 2 mg/ml did not induce DNA strand breaks, but hypercondensation of chromatin. Ponder these points that the hypercondenstion can be reversed exposure to light, and the concentration was much higher than that is used in veterinary medicine.[9]

If EtBr is really as dangerous as popular information are preaching, hospitals around the world would be crammed with molecular biologists suffering from cancer, or become superscientists. But this clearly is not the case.

Here are some statements verbatim from experienced EtBr users posted on reddit.com:

> Contrast this [use of tiny amount of EtBr in a gel] to formaldehyde, which your dissection specimen was probably swimming in and was probably all over your gloves the entire time.
> ~ shadyelf

> A PI (who ironically works in mutagenesis) was telling me how in the good old days of the 70/80s, she used to prepare gels using ethidium

> bromide without wearing gloves whilst smoking cigarettes!
>
> ~ monkeydustclive

> And of course, the cigarettes probably cause more deaths.
>
> ~ CerpinTaxtII

This story might be too far-fetched, but then, sounds genuine:

> I have professors that preach the EtBr safety protocols, yet they talk about handling the stuff bare-handed when they were in grad school; so much so that when they went out to the "disco" they're fingers would glow orange under the blacklights! These profs are still alive and each one that that I've talked to has normal kids with two arms and two legs, and one head.
>
> ~ haringsh [on protocol-online.org]

An advertising war?

The perpetrated taboo has led to a kind of a witch hunt among chemical companies creating a market hype that they have better and safer alternatives. On closer examination, these purportedly superior gel stains are not any more economical or safer. They are more expensive and sometimes bear hidden costs because of elaborate processing techniques.

Are the alternatives redundant?

Not necessarily, but it should be pointed out that each stain has its own advantage and disadvantage. PulseNet International has published an experimental comparison of EtBr with Gel Red, SYBR® Safe, and SYBR® Gold.[10] The report indicates that the alternative stains are not particularly superior to EtBr (Table 1). For instance, EtBr is relatively cheap, even if the surplus disposal cost is added. It is outstandingly stable after initial staining for several days. Its major drawbacks are its formation of background greying after prolonged exposure, and it has to be visualised under UV light. Perhaps, the most crucial issue is the quality of the gel bands, and all the stains are equally good.

Figure 4. Some adverts have really gone overboard.

There is a very negative investigation against EtBr by Chinese workers.[11] They simply compared SYBR® Gold, SYBR® Green, GoldView™, GeneFinder®, and GoldStar® as alternative to EtBr. They even recommended SYBR® Gold, which sound very much of advertising war. Their study showed that SYBR® Gold and SYBR® Green are not accurate for the fragment size of DNA in the gel, in indicating that they are not a preference to EtBr on that issue. Why did they come to such conclusion?

Are the safer stains safer?

Not necessarily, either. SYBR Green I stain and EtBr have been shown to show mutagenic activity in *Salmonella* strain TA98, although EtBr was more mutagenic.[12] On the other hand, SYBR Green I was much more genotoxic than EtBr on base-substitution mutations induced by UV-irradiation in *E. coli* B/r WP2 cells.[13] For the relief of SYBR Green II and SYBR Gold manufacturers, the two stains are quite safe in this respect and did not show mutagenicity either in frame-shift or in base-substitution indicator strains, TA98 and TA100, respectively.[14]

The case of SYBR® Safe is still elusive. According the Invitrogen brochure SYBR® Safe is "specifically developed for reduced mutagenicity, making it safer than ethidium bromide."[15] SYBR® Safe definitely has a safety advantage because it can be visualised using blue light transilluminator, thereby avoiding the use of potentially dangerous UV radiation mandatory for EtBr. Count your blessing, but pay an extra sum of money for the transilluminator. Is it any safer? The answer is quite debatable.

There is no real study of SYBR® Safe on its toxicity and mutagenicity, other than a non-reviewed report by Invitrogen.[15] According to the claim by MIT Green Chemistry Case Study that EtBr can be absorbed through the skin. Yes, it does. But it is poorly absorbed. While on the other hand, SYBR® Safe prepared with dimethyldulfoxide (DMSO) can be much more rapidly absorbed through the skin, because the high penetrating power of DMSO through tissues. DMSO itself is a rather inert compound, but because of its property it can act as a carrier for the stain into tissues. Remember that SYBR® Safe is a DNA-intercalating agent, same as EtBr.

In fact, Ward and Harper have carefully cautioned that even nitrile gloves are not safe for SYBR® Safe and its light sensitivity can be a problem while running a gel. They further suggest that "ethidium bromide or other nucleic acid binding dyes can be used as an alternative to SYBR® Safe".[16]

Figure 5. This ad entirely misses GelRed, which it is actually advertising.

AND THE PLOT THICKENS

We take antimalarials, quinine, chloroquine, mefloquine, antiviral drugs and antibiotics, for the treatment of the deadliest pathogens. These drugs kill the pathogens in us by destroying their nucleic acids, but save us. On similar vein, EtBr kills trypanosomes in cattle, saving the lives of cattle. I have wondered why EtBr would be any different in us.

There are real-life incidences to prove that EtBr is not necessarily harmful, and obviously not lethal or carcinogenic in humans.

The first taste

The Merck Index entry says that EtBr are "bitter tasting dark red crystals."[17] Someone had clearly and carefully tasted it.

The case of a strange coloured drink

Mei Cao *is* [the present tense is significant for the story, hence, my emphasis] a contented research associate in the Urology Department at the University of California, San Francisco. On two occasions, she noticed that her drinks were more bluish than usual. The first incident occurred on 23 October 2008 (some reports say 29 October).[18] But a thirsty person is no judge and simply gulped down the discoloured drinks without suspicion. It was only after a second incident that Benchun Liu, a post-doctoral researcher from China in the same laboratory, confessed to her that he tried to poison her. Cao immediately filed a case to the police, and Liu was arrested on charges of attempted murder.[19] Liu confessed to police that his choice of poison was, guess what, EtBr. That was why the drinks were discoloured. Liu had no defined motive for his action other than admitting that it was only because he was "stressed out." On fear for her life, Cao was taken to the university Medical Centre for thorough investigation. No abnormality, discomfort or symptom was found, and she was released as normal as was before.

Although Liu was accused of "poisoning and assault with a deadly weapon" in the San Francisco Superior Court, he pleaded not guilty, and no serious case came out of it. He could be right because the poison seemed to be quite non-poisonous. There is no further news of Cao of her health issues, nor a Super-Cao [pardon my coincidental and fictional analogy as in the earlier section, but I have every intention to claim full credit as the creator of these potential superheroes, if ever they become one]. There was not even the conviction of the failed murderer; in fact, he was released from prison on 25 November 2008.[20] On 30 March 2009, the prosecutors dropped the case because of "insufficient evidence to sustain the charges," and Liu went scot-free.[21] It was largely due to the legal expertise of Liu's attorney Bill Fazio, who decreed, "The substance wasn't a poison," and sympathised the defendant that "it was a pretty terrible strain on him and his family."[22] Yes, the *stain*! *The Scientist* ironically did not run out humour on the news, and concluded, "The lab's PI (Laurence Baskin), who studies cell signaling and bladder development, has refused comment. He's probably too busy checking all of his water bottles for a suspicious blue tint."[23]

I can vouchsafe on behalf of both the perpetrator and the victim that there was no further falling-out between them, as evidenced by their continued and joint publications in 2010 after the ordeal.[24,25] Cao is still a prolific author even today (Sinclair 16; overland).[26,27]

The failed suicide

There is a twist in another story. An anonymous person posted on the internet, in around 2007, that he/she was very concerned about a friend who tried to commit suicide by drinking and injecting herself with EtBr two years before, and whether or not she would develop health problem, particularly in having a baby. But clearly the suicide victim survived (two years is already quite long enough for EtBr to accomplish its lethal mission).[28]

These would disappoint Joshua, whose ideal suicide method would be to "bathe myself in a tub full of ethidium bromide so that my body would absorb enough of the stuff to ruin my DNA eventually leading to a slow death." I strongly do not recommend the dare or the internet site for this source.[29]

The moral of these stories has the reeks of the Garden of Eden's first total prohibition, "thou shalt not eat of it [of the fruit of the tree of the knowledge of good and evil]: for in the day that thou eatest thereof thou shalt surely die." Adam and Eve consumed that forbidden fruit. People had injected themselves with and drank EtBr. Despite the clear commands of death penalty in both cases, God was lenient, so is EtBr.

My take-home message is this: to commit a murder or suicide, EtBr is an ineffective choice of lethal weapon.

And the abbreviation EtBr reminds me of the undying words of the dying Caesar, "*Et tu, Brute?"* (a Latin for "You too, Brutus?", from

Shakespeare's *Julius Caesar,* 3.1.77) which I would love to charge upon my colleagues for their communal but irrational fear of handling EtBr.

By the way, there is no commandment which says, "Thou shalt not drink" – EtBr, alcohol or any type of poisonous liquid. But I implore all potential drinkers that they are not encouraged to do so.

For it is written: Touch not; taste not; handle not; which all are to perish with the using (Colossians 2:21-22a).

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