IN THE PIPELINE

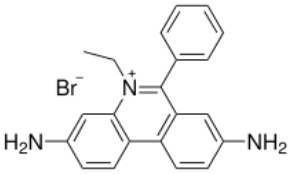
Derek Lowe's commentary on drug discovery and the pharma industry. An editorially independent blog from the publishers of *Science Translational Medicine*.

By Derek Lowe

The Myth of Ethidium Bromide

By Derek Lowe | April 18, 2016

Ethidium bromide is found in pretty much every molecular biology lab around. Ask most biologists about handling it, and you're get a fearful expression and advice to use gloves, etc. That's because the compound is used to make DNA fluoresce when **running gels**, and it does that by slipping neatly between the base pairs (intercalation), like sliding a card into a deck. That is not something you want to have happen to your own DNA, naturally, so EthBr is widely believed to be a human mutagen that should be dealt with cautiously. Laboratory suppliers certainly think so: a search for "ethidium bromide alternative" will bring up a whole list of "non-toxic, non-mutagenic" substitutes on offer.



There's only one problem with all this: ethidium bromide, as far as can be told from the data, is not a human mutagen. **It's not** a mouse mutagen or rat mutagen either. Nor apparently a mutagen in cows and other farm animals, where it's **used in veterinary medicine** at concentrations one thousand times higher than the red solutions that are so feared in biology labs, seemingly with no bad effects. It's not even **Ames-positive** by itself, but only after it's been exposed to metabolizing enzymes, which tells you that some derivative of it has mutagenic potential, should you ingest it and send it through your liver, but apparently not the parent compound. *(Note the 2002 "In the Pipeline" link – I've been doing this for a while, haven't I?)*

I write this as an organic chemist who handles worse stuff than EthBr all the time, and as someone who'd long been unable to grasp the molecular biology attitude towards the compound. You will see reference after reference to it as "highly toxic", "**notoriously unsafe**", and a "potent mutagen", when it's really none of those. These statements are bizarre, based on the amount of evidence behind them. **Here's Rosie Redfield** on the issue – she's also baffled by the attitude towards the compound, and has some questions about the safety profile of the alternatives that are being touted by other vendors and the over-the-top means used to deal with the compound itself:

> *Excessive concern about mutagenicity can make us overlook short-term toxic effects, and here EthBr is the safer dye. The reference above found that the SYBRsafe alternative was actually much more toxic than EthBr to the bacterial cells used in the mutagenicity tests. SYBRsafe was toxic at concentrations as low as 1 microgram/ml, whereas EthBr toxicity was not observed until 250micrograms/ml. The authors suggest that this is because living cells are much more permeable to SYBR green than to EthBr. But a **~~MSDS for SYBR safe~~** reports a LD50 for rats of >5g/kg, which is higher than that of EthBr (1.5g/kg). As both these LD50s are many orders of magnitude higher than the concentrations used in molecular biology, toxicity of gel staining solutions is trivial compared to the risks of of burns from melted agarose or slipping on spilled gel buffer.*
>
> *Perhaps the largest real hazards associated with use of EthBr in molecular biology are the methods used to inactivate it. Some labs now incinerate all waste containing even a trace of EthBr, and others absorb it onto activated charcoal. Harsher methods involve use of bleach and sodium hydroxide, or hydrophosphorous acid and sodium nitrite, all much more dangerous than EthBr.*

Yep, some of the "safe" alternatives actually light up the Ames test more than ethidium bromide itself. I'm not saying to bathe in the stuff, or use it to dye your hair. But it can be handled with *normal care* appropriate to a laboratory chemical, and not as the Mutagen From

look at the evidence – knowledge will protect you far more than fear ever can.

96 comments on "The Myth of Ethidium Bromide"

Morten G says:
April 18, 2016 at 8:02 am

What's wrong with absorbing it onto activated charcoal and sending it out with the other solid chem waste?

Derek Lowe says:
April 18, 2016 at 9:00 am

Is there any other waste that you treat like that, though? That still seems like overkill. . .

Sofia says:
April 18, 2016 at 1:12 pm

Honestly I could see scooping the whole gel slop into a solid waste container and spritzing the waste into aq. or organic waste depending on wash solvent – but you are right in that absorption onto carbon seems a bit bizarre.

Never the less I see people treat it that way because it is part of a SOP – and changing those is almost as difficult as changing opinions.

George says:
September 3, 2016 at 4:30 pm

Very true indeed

Morten G says:
April 19, 2016 at 6:27 am

Maybe there's more waste we should treat like that. I know that EDTA is a persistent environmental toxin that isn't degraded in normal sewage treatment plants. There are some places with plans to purify all the water that comes out of hospitals to remove drugs from the water system.

EtBr on activated charcoal is very easy though. You shine a blacklight at the sink once in a while and if it is starting to fluoresce you change the filter.

David says:
April 20, 2016 at 5:28 am

I am pretty sure you have eaten several times in your life EDTA, since it is a normal food ingredient. So being overly careful with lab waste because it contains EDTA, does not make sense.

Eric Johnson says:
April 21, 2016 at 2:36 pm

EDTA materials are routinely used in bulk fertilizer as a source of micro-nutrient metals (i.e. iron, zinc, copper, manganese and magnesium).

DK says:
April 21, 2016 at 1:10 am

Come on, guys, this is a very old news! Just read the original Ames paper. If memory serves, an extract from a single cigarette is 200 times more mutagenic than ethidium at concentrations well above those used to stain gels. Or, to put it another way, one would have to drink FOUR LITERS of typical gel staining solution to get mutagenic dose equivalent of smoking a single cigarette.

I smoked approximately 180,000 cigarettes in my life...

Paul Rohde says:

If you have a concern for EtBr and you think you need to absorb it on activated charcoal why would you do a procedure that concentrates it rather than leaving it diluted? When you use something in the fume hood it is diluted with more air (and sometimes diluted with a water scrubber.) Concentrating it is the worst thing that you could do.

John says:
April 18, 2016 at 8:08 am

The binding interaction between DNA and EthBr is often claimed to be pi-stacking, but I was under the impression that these interactions are usually perpendicular rather than the parallel geometry one expects when using the term "intercalator". Can anyone comment on this? I believe that parallel aromatic stacking only occurs between severely electron deficient and electron rich systems (EthBr looks to have intermediate electron density at a passing glance as it's a push-pull type system).

Random Scientist says:
April 18, 2016 at 11:02 am

From what I remember the aromatic interactions are largely driven by quadrupolar effects, which is I think what you are referring to when you mention the perpendicular interactions. This is how benzene interacts with itself, for example, by having the negatively polarized pi-electron cloud interact closely with the positively polarized C-H bonds.

When you insert charges and heteroatoms into the aromatic rings, however, this changes since the electron density in the pi-system is no longer uniform. With ethidium bromide having a positive charge, this should result in strong interactions with the negatively polarized pi-cloud of the DNA bases. But likewise, the variation in electron density of the pi-cloud caused by the heteroatoms in the DNA bases also allows all sorts of neutral aromatic heterocycles to interact strongly with the pi-cloud and stabilize these intercalating interactions.

AQR says:
April 18, 2016 at 4:45 pm

There is a very nice paper by Jeremy Sanders on "The nature of pi-pi interactions" (J. Am. Chem. Soc. 1990, 112, 5525-5534) where he analyzes both face-face and edge-face interactions to demonstrate how they are attractive. It's mostly a qualitative treatment and a quick read. I highly recommend it.

Andy says:
April 18, 2016 at 8:19 am

It feels like there's a gradual creeping increase in the hazards associated with most chemicals, but it's difficult to know whether they're the result of diligent testing and greater knowledge or the natural perverse incentives government regulation produces. For example, DMAP gets H310/H310, toxic if swallowed/fatal in contact with skin; it may be that DMAP is exceptionally toxic, but it never killed me despite using it many times and handling it ungloved more times than I care to remember.
It's not easy to see what incentive there would ever be for a regulatory body to reduce the described hazards associated with a compound, even if they had any reason to test and/or retest something. Why open yourself up to the potential action if the reassignment is superseded and the compound proves to be toxic again?

Biff says:
April 18, 2016 at 9:28 am

Based on the Prop 65 warning labels and signs you see in California, it seems that pretty much every hotel and restaurant in California is constructed of deadly, cancer causing agents. So silly.

bad wolf says:
April 18, 2016 at 12:14 pm

My wife and I noticed the same signs at Disneyland. Overkill indeed.

PS says:
April 18, 2016 at 9:53 am

I am not sure the notion that EtBr is dangerous was due to regulation. In fact the regulatory documents are pretty clear it is not. The craziness happens down the chain that implements the regulation, where every link tries to outdo the one above it. Throw in the mix somebody who has little clue what they are doing, except that they need to cover their a*s at any cost and anything can happen. For example last week I was told by our safety office that my lab's producers for handling lentivirus containing waste should be amended to include the following:

I guess the thinking behind this was something along the lines: Bleach is a good disinfectant, alcohol is also a disinfectant, so if we want to get the ultimate disinfectant we should mix them together.

Quintin says:
April 18, 2016 at 5:18 pm

So did you ask why you were supposed to be making chloroform?

Anon says:
April 18, 2016 at 8:42 am

Perfect example of how hubris takes over from real science and experimentation. We are back in the medieval times.

Biff says:
April 18, 2016 at 9:36 am

The amount of folklore in the practice of Science is astonishing. I did my graduate work in a well-known lab at a very prestigious institution, yet few people had ever bothered to read foundational papers in our field, and even fewer could explain why certain reagents were used and procedures were being performed in our lab. In many cases, our most routine activities were done with no evidentiary basis, but with the assertion, "that's how we've always done things."

Peter Kenny says:
April 18, 2016 at 10:17 am

One area in which the mythology really seems to have taken over is in the use of thermodynamic characterization of ligand-protein binding. We hear from self-appointed 'thought-leaders' that the enthalpy comes from the hydrogen bonds and that the entropy comes from the hydrophobic interactions. Voodoo thermodynamics perhaps? The mythology has become so pervasive that it is difficult so separate from opinion. I explore this theme in a post on PAINS that I've linked as the URL for this comment

Kramonikov says:
April 18, 2016 at 10:56 pm

Could you link to some of the foundational papers in your field?

Dave says:
April 18, 2016 at 8:43 am

This is something I have wondered for a long time as well. When I was a young graduate student I was taught how toxic ethidum bromide was and to handle it carefully. Years later, I had my own lab and taught my students and technicians the same thing. I hired a postdoc who came from Al Gliman's lab who was very caviler with handling ethidum bromide. I told him to be more careful and he told me that Dr. Gilman taught him that ethidum bromide was not toxic like everyone thought it was and to prove his point he picked up a ethidum bromide stained agarose gel with his bare hands and took a bite out of it. Apparently, Al Gilman had done the same thing when explaining to his students that ethidum bromide was safer than they thought it was. I did some research and found out that he was right. I don't make a habit of eating ethidum bromide stained gels but I also don't perpetuate the myth of the super toxic ethidum bromide.

KRL says:
April 18, 2016 at 10:17 am

Sounds just like the late, great Al Gilman that I knew.

Orle says:
April 18, 2016 at 12:35 pm

Al Gilman died from cancer. Did he eat too many EtBr-stained gels?

Cato says:
April 18, 2016 at 3:22 pm

Reminds me of Sharpless and osmium tetraoxide..

Tired Lecturer says:
April 18, 2016 at 4:24 pm

Biggie Mac says:
April 18, 2016 at 9:44 am

Most biologists know it is not likely to be all that toxic. Only 1st year students think it is carcinogenic and heaps bad for you lol.

PUI Prof says:
April 18, 2016 at 9:18 pm

I've got a department full of 'peers' that make me know you are incorrect in this assertion. And perhaps I shouldn't be belittling them. When a prof gets hung out by his university after his post-doc screws up, maybe I'm the one non-over-reactor who is wrong.

Paul Brookes says:
April 18, 2016 at 9:56 am

Agree EtBr is probably not a mutagen, but its mitochondrial effects should be enough to drive caution in its handling. In the mitochondrial field it's used in the generation of "rho-zero" cells, i.e. for the specific (?) depletion of mitochondrial DNA. As mitochondriacs, we treat it with the same caution and respect as as any other mitochondrial toxin (FCCP, rotenone, antimycin). None of these things if absorbed thru' the fingers in DMSO are going to kill you immediately, but it's not worth gambling on what might happen after a lifetime of exposure.

Anon says:
April 18, 2016 at 10:02 am

Speaking of proof vs hubris regarding toxicity, is there really any scientific proof for the toxicity of asbestos? I always wondered and doubted whether the stuff could really be that bad for you. Now I want to see proof.

zero says:
April 18, 2016 at 12:39 pm

Asbestos isn't toxic in a chemical sense (plain old silicates, nothing particularly exotic), but its physical structure causes disease when filaments lodge in lung tissue. A few thousand years of anecdotal evidence and nearly a hundred years of scientific investigation have established this conclusively.

Proper care allows the material to be used safely. Abraded particles are the risk, so sealed asbestos is generally safe. Construction crews routinely handle asbestos-bearing insulation during renovation of older buildings; use of masks, wet saws and proper air filtration is generally good enough.

We've eliminated asbestos from applications like brake pads where normal use would produce dangerous dust. People winning mesothelioma lawsuits today typically were employed in an asbestos industry and not provided with adequate training and protective gear. That is, negligence is the reason for the disease in most cases today just as negligence is the reason for most chemical injuries.

GravelInspectorAidan says:
April 20, 2016 at 6:53 am

There are at least THREE different silicate minerals which are used to make the industrial material "asbestos" ; they have considerably different health hazards.

That said, when Mum brought back some vein-asbestos samples from the rock wall of a car park at a major tourist attraction, I didn't bother to phone the council up and have the attraction shut down for 6 months. I don't lose sleep over it.

organic chemist still alive says:
April 18, 2016 at 10:09 am

I would say that the biologists have it pretty OK with their version of EtBr (ethidium bromide) versus chemists with their version of EtBr (ethyl bromide).

Boronsaur says:
April 18, 2016 at 10:43 am

When I was in grad school I used to be really scared of using HMPA, as the first thing coming to my mind was "HMPA is carcinogenic". Once I made a comment about this fear to my advisor and he reminded me that chloroform, a volatile solvent I still use for NMR, was also a carcinogen. So, proper ppe and reasonable care in handling HMPA or chloroform should keep me safe. My HMPA phobia went away.

A Nonny Mouse says:

When they were decommissioning the biological laboratories of the company where I used to work (whole site closure), the environmental people were going around at night with UV lights to determine potential spillages of ethidium bromide so that it could be dealt with properly. They must have made a fortune from this"toxic" chemical.

Anon says:
April 18, 2016 at 12:05 pm

Yeah, there is plenty of proof for the toxicity of asbestos. It got a lot of people badly sick in the past, and still can do so today.

That said.. it's no dimethylmercury, and it's dangers are exaggerated. The reason for said exaggeration is probably inspired by legal issues more than anything else.

Thomas says:
April 18, 2016 at 2:35 pm

The dose makes the poison. Asbestos can be handled double-bagged in multi kg quantities. I doubt many other poisons can be handled that way (carry that sack of cyanide please).

Still, it is insidious. And it has been promoted so much that there may have been backlash. But I am happy not to encounter it too much these days, being removed from everyday life. Which makes sense.

Strange that the asbestos businesses are still in operation. Proof that we protect business too much, I think. Ah well, tobacco.

food babe Vani Hari says:
April 18, 2016 at 12:24 pm

Chemicals are all bad.

Only use natural stuff, with no chemicals in it.

clawedfeet says:
April 20, 2016 at 3:06 pm

Just about all found in nature is chemical AND natural

I actually know what a chemical is says:
April 25, 2016 at 4:20 pm

Yeah, like that pesky dihydrogen monoxide!

Illapprove says:
May 6, 2016 at 6:37 pm

best bait I've seen, 10/10

Curious Wavefunction says:
April 18, 2016 at 1:01 pm

I remember using ethidium bromide with very few precautions as an undergrad. Sadly haven't sprouted wings yet.

dvizard says:
April 18, 2016 at 2:18 pm

Regardless of the actual mutagenicity, isn't also skin permeation of Ethbr actually relatively low? (Which was the lore that was floating around where I last worked with it. I never checked.)

Pingback: If it doesn't kill you... | Balanced Instability

Matt says:
April 18, 2016 at 3:44 pm

This folklore of fear reminds me of the weird terror people outside the explosives field seem to have regarding picric acid. Certain transition metal salts of picric acid are shock and friction sensitive. Picric acid itself isn't. It took almost 90 years after its discovery for its explosive properties to become known. It's insensitive enough to be fired out of artillery; it was a popular explosive filler for shells during

Wile E. Coyote, Genius says:
April 21, 2016 at 11:31 am

Look up the Halifax explosion. That was picric acid. However, most of the picric acid we use is in solution. It is when it crystalizes that it becomes an explosive hazard.

https://en.wikipedia.org/wiki/Halifax_Explosion

Matt says:
April 22, 2016 at 9:04 pm

Yes, picric acid is a high explosive, but it's not a *sensitive* high explosive. According to drop tests for mechanical initiation, it's about as sensitive as RDX ("Cyclonite") (see table XII here: http://www.dtic.mil/dtic/tr/fulltext/u2/116878.pdf) which is widely used in military explosive and propellant compositions. Like RDX, picric acid needs a very strong mechanic shock to detonate — shooting it with small arms won't work. Very large burning masses may self-accelerate toward the deflagration-to-detonation transition. That is not really an issue with laboratory quantities.

If the reason for paranoia is large accidental explosions in the past, people should be terrified of nitromethane too. If the reason is that it could become dangerously sensitive when contaminated with heavy metals, people should be terrified of sodium azide. In my experience, at least, people's fears of dry picric acid are disproportionate to anything documented in primary scientific literature or accident reports. If you drop a kilogram bottle of dry picric acid the biggest risk is that it lands on your toes and bruises them.

tangent says:
April 25, 2016 at 4:39 am

Old gauze pads with picric acid that have been in contact with a steel box under varying humidity?

Jeff says:
April 18, 2016 at 4:41 pm

A greater rat LD50 for SYBRsafe actually means it is less toxic than ethidium bromide (on a weight-for-weight basis) by at least 3 fold.

Hap says:
April 18, 2016 at 5:01 pm

Since the LD50 values for ethidium bromide and the replacement are so high (g/kg means the lethal human dose would be lots of grams), the toxicity margin there doesn't matter so much, while the enhanced cell toxicity for the replacement over ethidium bromide is problematic for its desired use.

Sydney says:
April 20, 2016 at 9:00 am

Jeff is right, higher LD50 means lower toxicity. The paragraph quoted in this article is misleading, implying SYBRsafe has higher toxicity because of higher LD50, but having a higher lethal dose actually means it is less toxic (takes more to kill).

Adrian Dingle says:
April 18, 2016 at 5:09 pm

Biologists think all kinds of strange things! https://www.adriandingleschemistrypages.com/ap/no-biologists-breaking-bonds-does-not-release-energy/

JSR says:
April 18, 2016 at 5:16 pm

I'm looking forward to a companion blog on the myth of toxicity from the minute quantities of radioactivity that biologists use in lab.

GravelInspectorAidan says:
April 20, 2016 at 6:58 am

Hey! Some of us have to explain the natural radioactivity of rocks to people on a regular basis. And then explain why the (gamma) radiation detectors that we use are not dangerous.

Don Atkinson says:
April 18, 2016 at 5:57 pm

It might not be all that terrible for you but if you are recovering a band from a gel to use in your expression construct then I strongly suggest that you use something, hell anything, else rather than EtBr. Unless of course you like sequencing a dozen clones.

And I really hope Al Gilman wasn't chomping on TBE gels because borate is not good for you at all.

hn says:
April 18, 2016 at 6:44 pm

EtBr may not be mutagenic, but the UV light used to visualize it is.

TheEdge says:
April 18, 2016 at 7:56 pm

You know, we chemists believe pyridine to be a male reproductive hazard, even with no evidence on the MSDS or in the reproductive hazard books I've seen. Anybody want to take on proving/debunking that?

R.K. says:
April 18, 2016 at 8:17 pm

This kind of structure reminds of me of irinotecan and topotecan a little. That phenyl group sticking out might be a problem, but it would be interesting to see whether it could also act as a stabilizer of the ternary complex (DNA topoisomerase unwinds DNA during replication/translation and those drugs stabilize it).

@ Matt: You should get in touch with the environment/health & safety people who work at our university. Our lab a few years ago found a small old unnoticed jar of picric acid, and of course the bomb squad had to be called in. That and a small fire have made the inspections an absolute pain in the *** ever since

Crimso says:
April 19, 2016 at 3:18 pm

EtBr is not a topo II poison, but if you make an azido derivative of EtBr and flash it onto the DNA, it is. The thinking was that topo II would cause the EtBr to dissociate wherever the enzyme was acting, but if you "locked" it in place it would have an effect. Related to the "positional poison" model work that involved abasic sites as topo II "poisons." See: "Covalent attachment of ethidium to DNA results in enhanced topoisomerase II-mediated DNA cleavage." Marx G, Zhou H, Graves DE, Osheroff N. Biochemistry. 1997 Dec 16;36(50):15884-91.

Dan Schindler says:
April 19, 2016 at 4:20 am

Re: "The authors suggest that this is because living cells are much more permeable to SYBR green than to EthBr"

Thermofisher
Propidium iodide (PI) is a popular red-fluorescent nuclear and chromosome counterstain. Since propidium iodide is not permeant to live cells, it is also commonly used to detect dead cells in a population.

Tony Ashton says:
April 19, 2016 at 6:51 am

Yes Dan, the use of propidium iodide (PI) as a stain for the DNA of dead cells but not living cells surrounded by an intact plasma membrane suggests that the closely related ethidium bromide (EB) also does not enter living cells. PI will dissociate in solution into the positively charged, impermeable propidium ion and negative iodide. Similarly EB becomes positively charged ethidium and Br-. If the propidium and ethidium ions can't enter intact cells then toxicity by intercalation with DNA is improbable. Perhaps someone who does PI staining should run an EB control.

Hugh Fletcher says:
April 19, 2016 at 7:08 pm

Caffeine is mutagenic, radiomimetic, and interacts with DNA in a similar manner to ethidium bromide. see for example CANCER RESEARCH 28, 2375-2389, November 1968

There can be 200 miligrams of caffeine in a cup of coffee, compared to a few micrograms (about 1/10,000th. the concentration used to stain gels. Drinking coffee is more dangerous than sticking a healthy finger into gel stain. Ethidium at that concentration won't get through the skin. Some people like the taste of coffee, I suppose that is why they do it.,

Curt F. says:
April 20, 2016 at 12:20 am

I confess! I was one of the sheeple that learned that ethidium bromide was very toxic and have actually (probably mistakenly, I now realize) to use nitrite/hypophosphite decontamination procedures.

1. Why do we always use ethidium bromide instead of say ethidium chloride or ethidium nitrate? Is the bromide salt that much easier to prepare?

2. In my microbiology days I was always puzzled by protocols and SOPs that claim kanamycin is "light sensitive". Does that make sense for what is effectively a diamino-trisaccharide? I'm not a card-carrying chemist, but I don't see any obvious photophores in the molecule. Is this another biology myth?

slava bernat says:
April 21, 2016 at 10:00 am

Could someone confuse kanamycin with kinamycin?

Teemu says:
April 20, 2016 at 5:03 am

I am a plant molecular geneticist and together with many colleagues have the opinion that the public opinion about genetic engineering in agriculture (GM crops) shows ignorance and lack of straight thinking. Agriculture is seen black-and-white, GM is bad and non-GM is good. But we at the same time show exactly the same thinking about lab chemicals: If you protect yourself from EtBr by wearing gloves etc., you are safe. EtBr seems to be the only dangerous chemical, or at least the worst chemical in the molecular biology lab.

G. says:
September 3, 2016 at 5:28 pm

Well, although I'm a little concerned about releasing GM-crops in the fields, rather than eating them, I do agree that we often get obsessed about EtBr (I remember a pregnant colleague did not want to enter the room where two years before EtBr was in use-and since then it was stopped and properly disposed and cleaned), while at the same time we might be fine with leaving open bottles in the chemical hood with the cover open...
There is certainly a lot of information that go from supervisor to students over generations, as it is, but on the other hand, better be safe than worried...

Pingback: The Myth of Ethidium Bromide | In the Pipeline | QUANTUM BIONOMICS

Adarsh Gupta says:
April 20, 2016 at 9:43 am

I do not know how this dubious piece of writing could show up in sciencemag. Not all mutagens need to show up positive in Ames's test, no matter how mutagenic they actually are! I have seen great scientists die of cancer; be it because of EtBr (and other intercalating agents) or UV radiation (Pyrimidine dimerisation) or radioactive isotopes (and other sources of ionising radiation), molecular research is undoubtedly hazardous. We need to be more careful not less, and it's better to be safe than sorry!

Brian Crawford says:
April 20, 2016 at 12:02 pm

Odd article. I wonder about the definition of a posltive Ames test? Certainly 2 seconds on google reveals this paper:
http://www.ncbi.nlm.nih.gov/pubmed/10029672

In the abstract:
"As expected [J. McCann, E. Choi, E. Yamasaki, B.N. Ames, Proc. Natl. Acad. Sci. USA, 72 (1975) 5135-5139], ethidium bromide showed high revertant frequencies in several frameshift indicator strains (averaging 68-fold higher than vehicle controls in TA98, 80-fold higher in TA1538, 15-fold higher in TA1537, and 4.4-fold higher in TA97a)".

Also, in the 1975 paper (J. McCann, E. Choi, E. Yamasaki, B.N. Ames, Proc. Natl. Acad. Sci. USA, 72 (1975) 5135-5139) Ethidium Bromide is classified as a mutagen i.e. positive for the Ames test.

Can the author of this blog respond? This blog post should be corrected or deleted.

April 22, 2016 at 12:53 pm

Congratulations on using Google to independently find a paper cited and discussed in Rosie Redfield's [2006!] blog post that was partially quoted here.

Also, you omitted "only in the presence of rat liver extracts (S9)" from the end of the sentence you quoted from the 1999 Molecular Probes [now Invitrogen] abstract.

Xiang Li says:
April 22, 2016 at 2:12 am

We just happened to do a test recently – A plasmid was run on agarose gel with SYBR safe. Then the gel was exposed to UV (312nm), and then the plasmid band was cut out and recovered by Qiagen gel extraction kit, quantified and compared transformation efficiency side-by-side with the same amount of the same plasmid DNA. The plasmid DNA exposed to SYBR safe and UV has no transformants at all, while the one without any treatment gave lots of colonies as expected. When combined the treated DNA with the untreated plasmid, it dramatically reduced the transformants number. It seems very toxic to the competent cell. From this test it raise the concern – with the permissive of SYBR safe to the cell, if it bind to DNA, when exposed to UVB, is it toxic or not? Can SYBR safe and DNA cross link under UV? Hope someone can explain the possible chemistry.

Tarik Dinc says:
April 22, 2016 at 4:17 am

in that uv energy SYBR Safe can react give dielsalder reaction with nucleotides and add to nucleotides

Alf says:
April 22, 2016 at 10:39 am

Speaking of myths in safety regulations, there's a wide interval of strictness in handling of retro/lentiviral construct for gene transfer and safety for operators. In my lab, we started using 3rd-generation lentiviral supernatants from transfected packaging cells under BSL3 conditions, we then switched to BSL2 as our facility was downgraded. I struggle at times to have titers of 10E5 particles/ml which would then infect some cancer cell lines at a decent rate, while others would be more resistant to infection, and I often wonder whether this viral supernatants (replication incompetent, being devoid of all the replication machinery) would represent a biological risk for operators. Yet, all the operators are extremely cautious handling them. Besides safety practises, is there any reference to the actual risk of infection for operators?

Colin Garner says:
April 22, 2016 at 12:04 pm

Another barely genotoxic compound is dioxin – maybe we should give that a break too?

Aude@ANU says:
April 29, 2016 at 9:41 am

Thank you for your article.
I too realised that EtBr was not mutagenic after unsuccessfully trying to use it as a positive control in an Ames test for an undergraduate lab. I then looked up Ames' original papers: indeed it is not a mutagen.
An older colleague pointed me to the Merck index which includes an entry under taste (bitter).
Turns out it was used as a food dye for years.
The hysteria by laboratory regulators is extremely dangerous: as safe chemicals are all labelled as highly toxic, a new student has no way of knowing which chemicals really are dangerous. Instead, they start to assume that all safety warnings are rubbish. The problem is that a small minority of warnings are very very real.

Venkatesh says:
July 13, 2016 at 12:36 pm

Well, that makes people who have already proved EtBr to cause frameshift and point mutations as morons?

Arduenn says:
April 30, 2016 at 12:06 am

I'd worry more about exposed wrists above the UV lamp when cutting out DNA bands from EtBr-stained gels.

Cristina Pungartnik says:

Something that does not kill immediately does not mean it is not harmful or dangerous.
EtBr is proved to be mutagenic (induces mutations) in Ames test after metabolization (that means, after P450 enzymes metabolizes and transform it to the mutagenic substances, which would be similar to what our liver does).
"J. McCann, E. Choi, E. Yamasaki, B.N. Ames, Proc. Natl. Acad. Sci. USA, 72 (1975) 5135-5139], ethidium bromide showed high revertant frequencies in several frameshift indicator strains (averaging 68-fold higher than vehicle controls in TA98, 80-fold higher in TA1538, 15-fold higher in TA1537, and 4.4-fold higher in TA97a), only in the presence of rat liver extracts (S9)." https://toxnet.nlm.nih.gov/cgi-bin/sis/search2/f?./temp/~OuLeBc:4
Accordingly to recent research "Safety data about ethidium bromide (EtBr) are contradictory" and conclude that it is toxic and mutagenic in yeast eukaryotic system Saccharomyces cerevisiae. Yeast. 2015 Sep;32(9):595-606. doi: 10.1002/yea.3081. Epub 2015 Jul 21.
Also, it has been shown with the Frog Embryo Teratogenesis Assay: Xenopus (FETAX) teratogenic and growth-inhibiting potential of DNA, RNA, and protein synthesis inhibitors induced by EtBr. Teratog Carcinog Mutagen. 1985;5(3):177-93.
Storage condigions and handling safely has long time ago been ruled and should not be forgotten. https://toxnet.nlm.nih.gov/cgi-bin/sis/search2/f?./temp/~OuLeBc:3 . Which includes "Ethidium bromide should be handled in the laboratory using the "basic prudent practices" described in Chapter 5.C. Because of its mutagenicity, stock solutions of this compound should be prepared in a fume hood, and protective gloves should be worn at all times while handling this substance. Operations capable of generating ethidium bromide dust or aerosols of ethidium bromide solutions should be conducted in a fume hood to prevent exposure by inhalation. [National Research Council. Prudent Practices in the Laboratory. Handling and Disposal of Chemicals. Washington, DC: National Academy Press, 1995., p. 310] **PEER REVIEWED**".
We do not expect any of our students to drink or eat enough EthBr to go immediately to the hospital. However, long time exposure (years of working in the lab to conclude their graduation, master, PhD, pos-doc and then, finally being a supervisor) makes them a group of workers in long term more constantly exposed to harmful chemicals and possible accidents in the lab, and thus, professors and supervisors should be more careful when giving instructions how to deal with EtBr or any other chemical substance in the lab. That is part of something called Risk assessment (determination of quantitative or qualitative estimate of risk related to a well-defined situation and a recognized threat, also called hazard).
Certainly it is not a chemical "regarded as safe" and should be therefore handled with appropriate care in any kind of lab.
Afterall, a swiss alchemist and physician Paracelsus (1493–1541) had already said: "All substances are poisons; there is none that is not a poison. The right dose differentiates a poison from a remedy."

Venkatesh says:
July 13, 2016 at 12:33 pm

These days people believe more in shitty blogs rather than research works. Funny

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Lee Price says:
July 18, 2016 at 9:26 am

This blog's author has made his credentials and academic work public. What is it that YOU do, exactly?

Imer says:
July 15, 2016 at 11:52 pm

So I'm at this college program and we ran and gel electrolysis. Afterwards, I had taken off my gloves because we had done like an hour of waiting and I accidently touched the gel. I immediately washed my hands and showered when I got back to my rooms but I am still very concerned. What should i do????

Virginia says:
August 18, 2016 at 10:18 am

How about the vapor generated when you add EtBr to the agarosis still hot (temperature around 37C)? Most people that I know are really concerned about it too.

---

marco casteleijn says:
August 31, 2016 at 12:59 pm

As far as I understood it act as a neurotoxin and goes in the skin pretty easy. Does it not degrade the in myelin-sheets giving you shaky hands over periods of long exposure?

Derek Lowe says:
August 31, 2016 at 3:51 pm

None of the above. It is not a known neurotoxin in any species I'm aware of, and does not seem to penetrate the skin – it just stains the dead cells on the surface (it doesn't stain live cells, because it doesn't penetrate intact cell membranes).

Marco, you might be thinking of acrylamide, the monomer used to make protein gels.

Adam says:
September 1, 2016 at 11:10 am

A former supervisor of mine said there was a case in France where the entire lab were diagnosed with cancer. Upon inspection, there was found to be EtBr everywhere. Does any one know of this case?

Nick Bauer says:
September 1, 2016 at 1:27 pm

Was EtBr the only thing found? Because I can easily imagine that if lab practices were sloppy, there could easily be other compounds spread around as well.

Mariana says:
September 1, 2016 at 10:55 pm

You are kidding about not dying the hair, but I found this compound in the ingredients of the dye Garnier about 10 years when my mum was using it. I told her and explained. I send a mail to Garnier with no response. Later I was looking for the ingredients in the next month and nothing..I guess they were hidden the information.

G. says:
September 3, 2016 at 5:46 pm

What I was always wondering is that, if EtBr is dangerous because it binds to DNA, all the DNA-binding dyes would be more or less the same. And low permeability doesn't mean no permeability, right?
Of course it's all about probabilities...
But then considering all the dangerous chemicals we use...

John Campbell says:
September 3, 2016 at 6:14 pm

Adam, it was at the Pasteur Institute in Paris back in the mid 80s. Six people in two labs developed different cancers over a two year period. Various reagents were blamed without good evidence. See here. http://www.sjweh.fi/download.php?abstract_id=61&file_nro=1

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Meher Hassan says:
February 23, 2017 at 2:35 am

It feels like there's a gradual creeping increase in the hazards associated with most chemicals, but it's difficult to know whether they're the result of diligent testing and greater knowledge or the natural perverse incentives government regulation produces. For example, DMAP gets H310/H310, toxic if swallowed/fatal in contact with skin; it may be that DMAP is exceptionally toxic, but it never killed me despite using it many times and handling it ungloved more times than I care to remember.
It's not easy to see what incentive there would ever be for a regulatory body to reduce the described hazards associated with a compound, even if they had any reason to test and/or retest something. Why open yourself up to the potential action if the reassignment is superseded and the compound proves to be toxic again?

Pingback: Is Ethidium Bromide Safe? - The Scientific Student

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