”Cobalt Buster" Project :

# Safety

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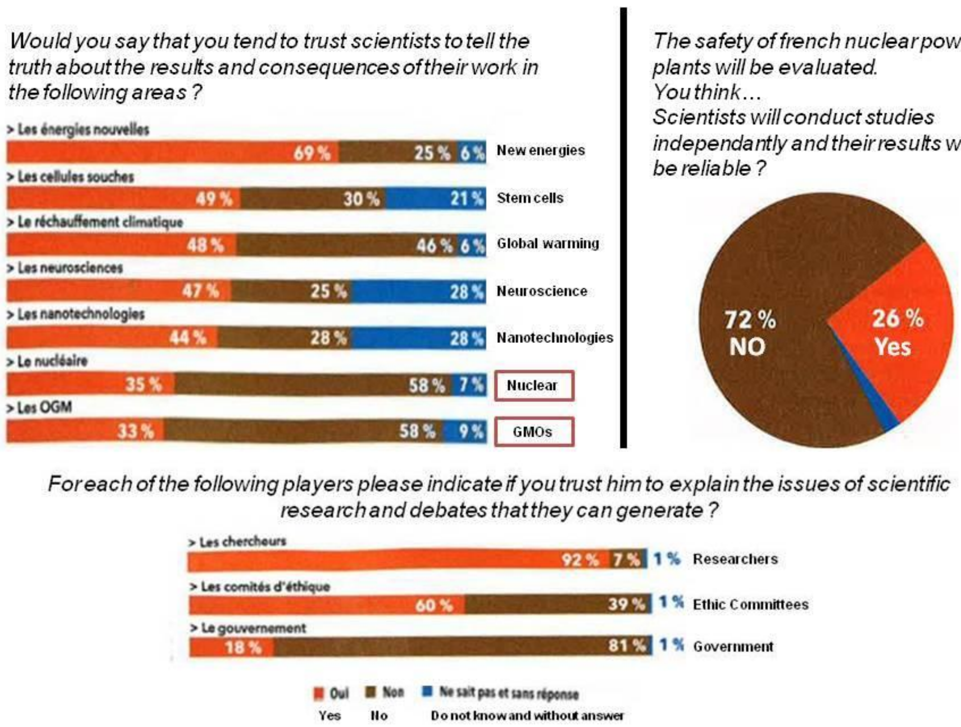
# Introduction

We present here our reflexion about safety issues of the "Cobalt Buster" project based on a modified*Esherichia coli* strain able to capture and concentrate cobalt from its environment. **This reflexion based on the questions provided by iGEM safety judges**, is presented in a form that we believe more convenient.

Radioactive cobalt is released in water systems of nuclear power plants, that's why we aim at using **this strain as bio-filter** for nuclear wastewater treatment to **improve efficiency** and **reduce both nuclear waste volume and the costs of the treatment**.

In this context we considered Researcher safety and, as we are aware that this project combines two technologies that scare a significant proportion of the population (GMO and nuclear power plant, as shown by a recent french survey resumed below), we paid particular attention in defining the potential risks for the Public and the Environnemt.

French survey published in « La Recherche » n°455 in september 2011




**To prepare safety issues we organised a public debate called [" Nuclear technology and Genetically Modified Organisms : Can scientists keep control? "].**

# Researcher Safety

First of all, **no radioactive elements** will be manipulated during this project. To ensure safety of students during lab handling, only non radioactive cobalt will be used.

All *E. coli* strains we used have a **biosafety level of 1**, which means they are not known to cause diseases and have minimal environmental hazards. Although the final "Cobalt Buster" strain is designed to work in radioactive environments, it can be produced in normal conditions, like any other strain.

**Cobalt** is **toxic by inhalation** and **contact** and must be **manipulated with gloves and masks** and disposed in appropriate waste containers.
**DNA manipulation** will require the use of solvents and carcinogenic molecules which require the use of **gloves and chemical hoods**.

Thus, as for every bio-synthetic project, usual **lab safety measures** are enough to protect researchers efficiently during the "Cobalt Buster" project : wear a labcoat, gloves and dispose biological material in biohazard containers and metal in specific container. This strain is not more dangerous for people in the lab than any other E.coli strain, and thus doesn't require any additional care in handling them.

# Public and Environmental Safety

We considered Public and Environmental safety from both hazard and probability point of view. To measure how **nuclear power plants related procedures** and **nuclear waste treatments** are strictly regulated, we organised **two visits**. First we visited **the nuclear power plant of Tricastin** to have an idea of **how radioactive compounds are confined** and how **human workers are protected**. Then we chose to visit **the Centraco** site which attend to a part of radioactive waste management.

## Hazard

In usual working conditions, **our strain will accumulate radioactive cobalt**. Cobalt is **toxic** by inhalation and contact. It has been proven to *cause cancer, respiratory system damage, skin damage*among others on humans and various effects on other species including plants, [1] . It is important to notice that cobalt in our bacteria could be more concentrated than in usual resins. Radioactivity of the compound adds to the danger, with various damages that can't be neglected : *nausea, cerebral edema, sterility, foetal damage...* Only low-dose effects can be effectively treated. A previous study [2] showed that **cobalt accumulation capacity of the bacteria** is **not infinite** and that bacteria death will conduce to**the liberation of cobalt** in the medium with the potential health or environmental issues that have been described if it ends up in the environment. For these reasons it is very important to ensure that the"Cobalt Buster" bio-filter will not release bacteria in environment after the processing.

**In case of an unexpected release of the bacteria** in the environment before it has fixated cobalt, we consider that **hazard is low**. Indeed, adding to the *difficulties for the bacteria to survive* in the Environment, none of the parts we will construct present a direct danger for Public as they will not modify the biosafety level of the *E. coli* strain and the bacteria does not produce any human or environmental toxic element by itself. However, *antibiotic resistances* carried by the different parts we will add to the *E. coli*strain may provide a selective advantage in environments where antibiotic selective pressure is high. Antibiotic resistances could be transferred to other bacteria strains, potentially human pathogens which would be favored by natural selection in such environments.

As far as **malicious use** is concerned, this strain could indeed be used to capture and concentrate cobalt from a medium in order to use it to pollute water for example. However, this method is more complicated and less efficient than using other poisons in liquid solutions, which makes it **a very poor way of causing intentional environmental or health troubles**.

**If a serious nuclear incident occurs** (as INES scale level 7 nuclear disaster), the presence of our"Cobalt Buster" bio-filter will **not enhance adverse consequences on health and environment**. Indeed, in this case radioactivity level of the bio-filter can be neglected compared to releases generated by the incident and bacteria will probably be killed.

| Type | INES | Impact on People & environment | Impact on Radiological Barriers and controls | Impact on Defence-in-depth |
|---|---|---|---|---|
| Major Accident | 7 | Major release of radioactive material with widespread health and environmental effect requiring implementation of planned and extended countermeasures | / | / |
| Serious Accident | 6 | Significant release of radioactive material likely to require implementation of planned countermeasures | | |
| Accident with wider consequences | 5 | Limited release of radioactive material likely to require implementation of planned countermeasures<br>Several deaths from radiation | Severe damage to reactor core<br>Release of large quantities of radioactive material within an installation with a high probability of significant exposure | |
| Accident with local consequences | 4 | Minor release of radioactive material unlikely to result in implementation of planned countermeasures other than local food controls.<br>At least one death from radiation. | Fuel melt or damage to fuel resulting in more than 0,1% release of core inventory<br>Release of significant quantities of radioactive material within an installation with a high probability of significant public exposure | |
| Serious incident | 3 | Exposure in excess of ten times the statutory annual limit for workers.<br>Non-lethal deterministic health effect (e.g., burns) from radiation | Exposure rates of more than 1Sv/h in an operating area.<br>Severe contamination in an area not expected by design, with a low probability of significant public exposure. | Near accident at a nuclear power plant with no safety provisions remaining.<br>Lost or stolen highly radioactive sealed source.<br>Misdelivered highly radioactive sealed source without adequate procedures in place to handle it. |
| Incident | 2 | Exposure of a member of the public in excess of 10 mSv.<br>Exposure of a worker in excess of the statutory annual limits. | Radiation levels in an operating area of more than 50 mSv/h.<br>Significant contamination within the facility into an area not expected by design. | Significant failures in safety - provisions but with no actual - consequences.<br>Found highly radioactive sealed orphan source, device or transport package with safety provisions intact.<br>Inadequate packaging of a highly radioactive sealed source. |
| Anomaly | 1 | | | Overexposure of a member of the public in excess of statutory annual limits.<br>Minor problems with safety components with significant defence-in-depth remaining.<br>Low activity lost or stolen radioactive source, device or transport package. |
| Deviation | 0 | | | No safety significance. |

However, according to this description, we can estimate the type of incident directly linked to « CobaltBuster ».

The major problem which can occur with our bacteria is the dispersion in environment, for example by a water leak. This type of incident should be classified in Incident (level 2) or Serious Incident (level 3 - "Severe contamination in an area not expected by design, with a low probability of significant public exposure). Indeed, the public exposure would not be important, because the radioactivity of our bacteria is low and the dilution in the river is quite important. Problems during the manipulation, the treatment and the transport of the filters can induce incident of level 1 or 2, depending on the intensity of the radioactivity of the filter.

We can see that our "CobaltBuster" should be used with high precaution, to avoid her dispersion and the contamination of the environment.

All this hazard is mainly caused by the capacities of accumulation of cobalt of the "Cobalt Buster"strain, added to the ease of retrieval thanks to the adherence. The parts that we are creating allow to make any *E. coli* strain adherent in presence of cobalt, so **no part we are going to enter to the registry can be considered hazardous in a regular *E. coli* strain**

# Probability

Once in working conditions, our strain will **form a biofilm** and be **bound to a confined filter**. As the biofilter is intended to work in nuclear power plants to capture radioactive cobalt, every steps of the industrial use of the "Cobalt Buster" strain will be done in **confined conditions with a very strict procedure**. Indeed, after the capture of radioactive cobalt in nuclear waste-water, our "Cobalt Buster"filter will be considered as **nuclear waste** and it will be supported following a very strict and highly regulated procedure.

Strict radioactive discharge protocols and storage conditions ensure that **the probability of unintentional release is close to zero**. Nuclear power plants are extremely confined and regulated areas. This implies that the probability of an unexpected event is extremely low. Moreover, water systems treated by the filter are isolated from each other and especially isolated from the environment which greatly reduces the risk of release.

All procedures in place in nuclear power plants are made **to respect the precautionary principle** and**reduce the exposure of humans and environment to the minimum**. The presence of our "Cobalt Buster" bio-filter will not increase the probability that a nuclear incident occurs in the power plant. We must notice that only two major nuclear incidents have occurred in the last fifteen years of nuclear power plants exploitation : is it low or high incident probability ?

# Conclusion

As a conclusion, despite the danger due to the accumulation of radioactive metal in a non pathogenic *E. coli* strain, the potential hazard is rather low compared to other damage that an accident in a nuclear power plant would produce. Moreover, our device would change very little to the processes already implemented in nuclear power plants, that work with minimum exposure. Confinement in nuclear areas ensures a very low probability : the discharge is thoroughly controlled. Malicious uses are extremely unlikely due to the presence of more efficient ways of achieving the same result. **The potential benefits** of the "Cobalt Buster" bio-filter, **reducing the volume of nuclear waste by 100** and **decreasing costs of waste disposal**, are **greater than the risk we run**, and according to us justifie the addition of such a device in nuclear power plants.

References :

[1] Cobalt et ses dérivés, INERIS, 2006 april, available here

[2] Bioremediation of trace cobalt from simulated spent decontamination solutions of nuclear power reactors using E. coli expressing NiCoT genes. Raghu G, Balaji V, Venkateswaran G, Rodrigue A, Maruthi Mohan P. Appl Microbiol Biotechnol. 2008 Dec.

# Biosafety Guidelines

Neither of our institutions (INSA Lyon and ENS Lyon) have a **biosafety group**. However, we have a general safety and health committee that deals, among others, with issue related to GMOs and that allowed their handling in the different institution, that has however not reviewed our project. All students follow a 4 hour **general health and safety lecture**, teaching how to handle chemical, biological and fire risks among others, completed by **additional biosafety and lab training all along the year** by the professors, in relation to their course. Our institutions do not have any specific biosafety rules and follow the general french laws on biosafety.

As far as the legal aspect is concerned, **synthetic biology doesn't have specific rules** yet in France. As our bacteria are Genetically Modified Organisms, we are due to respect the **general laws** about the use of GMOs and **ethics**, which are relatively restrictive in France, based on the the precautionary principle. And even if synthetic biology doesn't have a specific regulation nowadays, the French government begins to think about it. Indeed, a reflexion is on-going in the National Assembly to measure all the possibilities of Synthetic Biology and define what can be allowed and what should be forbidden. A first congress and public audition (Program) occurred in May. One of our instructors made the trip to the public audition. You can read the conclusions here. To sum-up, it appears that Synthetic biology is a useful way to answer to future challenges. However, to avoid a miscomprehension from public like with the GMOs, it's necessary to improve communication and education.

In industrial conditions, additional safety rules about handling radioactive material will need to be applied because of the accumulated radioactive cobalt in the bacteria : confinement, limitation of human exposure, storage in adapted radioactive waste containers ensuring that no cobalt escapes to the environment during its lifetime, control of the composition of the water liberated into the environment... These rules are already implemented.

# For a Safer Genetic Engineering ?

From the debate **"Nuclear technology and Genetically Modified Organisms : Can scientists keep control?"** we organized, several safety issues were risen and suggestions have been proposed to tackle them.

First, **the standardisation of parts** makes it simpler to **use and share for researchers**, but also **for malign or careless uses**. All the informations related to the iGEM projects are freely available on the Internet **without any access control**. It has been evoked that, with these informations, anyone with basic microbiology knowledge could try to build **his own bio-weapon** (like people nowadays can find the recipe to create their own bomb on the Internet) or, on a safety point of view, could **misuse a part and accidentally harm people or environment**. *Yet, would it be a better solution to restrict access to iGEM members ?*
It would mean to give up on the valuable "open source" model, where everyone's experience contributes to enhancing our knowledge, including the knowledge about the safety of the parts (what should and should not be done with a part, what precautions you must take, what unexpected behavior has been observed...). Concealing the information would not prevent people from accessing it illegally, as shown by the numerous web security breaches that are regularly reported. This means that synthetic biology should be careful about safety issues, and discussions or reflections concerning the "open source" should not be overlooked.

**General safety issues about GMOs** have also been mentioned, and the reasons why the general public considers them unsafe. The quick development of plant GMOs was, in the eyes of the general public, a search for immediate profit with few concerns about safety and ethics. And moreover very few efforts of communication have been made, which has created a prejudice about GMOs. We noticed that generally, **GM bacteria** are widely used in **medicine and food industry**, but are less known by the public who is less scared about them. Synthetic biology should avoid such a mistake, by communicating to the public before releasing new devices, and ensuring that they will be accepted by a distrust public.

We are not alone to think about all these considerations. A great debate will occur at the occasion of the European Jamboree in Amsterdam in the aim to define an **Oath for Life Scientists**.