

# focus on Chromatography

## The Importance of the Analysis of Nitrosamines by a Thermal Energy Analyser (TEA) and Their Link to Cancer

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Many cancers have been attributed to the exposure of nitrosamines or nitrosamine forming compounds. Other cancers may be caused by the metabolism of nitrates and nitrites to form nitrosamines within the body.

Nitrosamines are one of the most studied sets of carcinogenic compounds but the final mechanisms from the presence of a nitrosamine to the uncontrolled growth of effected cells is not fully understood. Different nitrosamine compounds have different levels of toxicity and attack specific organs within the body.

Nitrosamines can enter the body orally through consumption of food and drink or continued oral contact with products such as baby pacifier's or toys. As many of the nitrosamines are volatile, aspiration is a possible intake mechanism into the lungs. This form of intake can be either voluntary in the case of smoking tobacco products or involuntary in terms of breathing atmospheres laden in nitrosamines from certain manufacturing processes.

Nitrosamines are compounds with the general structure  $R'N(R'')N=O$  (Figure 1) and the vast majority are deemed to be carcinogenic. There are many different methods of formation for nitrosamines. Where heating occurs in the presence of a secondary amine and  $NO$  ie nitrates/nitrites, nitrosamines can readily occur. Nitrosamines have also been observed to form under strong acidic conditions, such as the stomach.

There are generally three main groups of nitrosamines which are commonly found; these are the volatile, tobacco specific and non-volatile. The volatile nitrosamines generally possess low boiling points, the most commonly analysed is n-nitrosodimethylamine (NDMA), which has been shown to have the worst detrimental effects on human health. The tobacco specific nitrosamines (TSNAs) are found in tobacco and also formed during the burning process. The most commonly analysed TSNAs are formed from naturally occurring compounds such as nicotine, anatabine and anabasine which are present in the tobacco leaves. The non-volatile nitrosamines are often found in food stuffs being adducts from larger molecules like amino acids and nitrites.

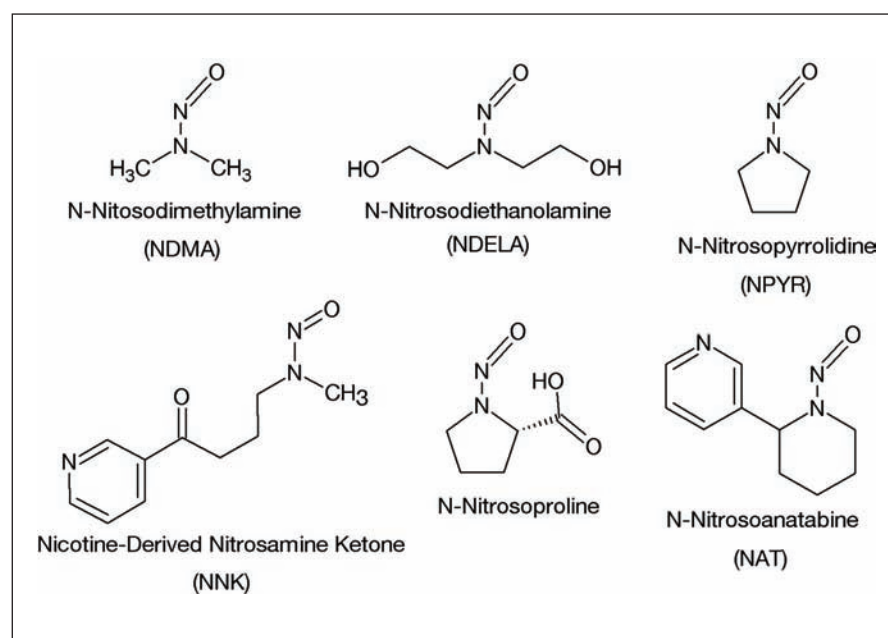


Figure 1. Chemical Structures of Nitrosamines

The TEA has been the detector of choice for analysing nitrosamines for many years. Connected to either a Gas Chromatograph (GC) (Figure 2) or Glassware Chemical Stripping system (Figure 3) the detector is able to provide the selectivity and levels of sensitivity required for the analysis.



Figure 2. Ellutia 800 Series TEA connected to a GC

### Known Sources of Nitrosamines:

#### Tobacco Products

Tobacco is one of the more commonly known products that has the proven side effect of causing cancer of lungs, throat and mouth. The four compounds thought to be responsible for these cancers and routinely measured are: n-nitrosornicotine (NNN), nicotine-derived nitrosamine ketone (NNK), n-nitrosoanabasine (NAB) and n-nitrosoanatabine (NAT) (Figure 4). NNN and NNK have persistently been proven to cause cancers in laboratory animals with NAB showing a degree of weak carcinogenic effects and NAT proving to be the least active of these compounds.

Traditionally tobacco products would include cigarettes, cigars, snuff and chewing tobacco, but recently alternative ranges of tobacco products are available for customers concerned about health issues, including smokeless cigarettes, gums and low nicotine cigarettes. Although these newer products overall contain less of the carcinogens, some contain as much as the light or ultra light standard cigarettes, so it is still to be seen if these reduce the risk of cancer.

Responsible manufacturers of tobacco products try to limit the levels of TSNAs in their products and adhere to government guidelines and test batches of all their materials.

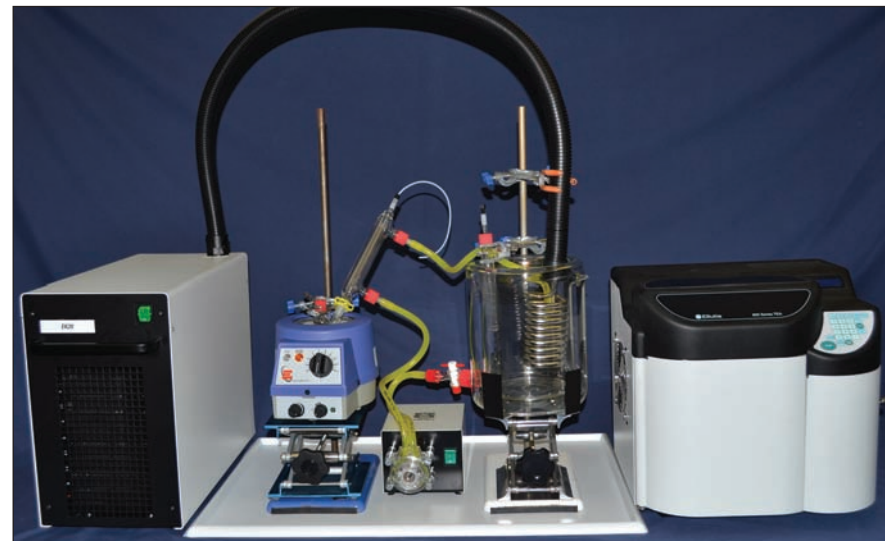


Figure 3. Chemical Stripping System with an Ellutia 800 Series TEA

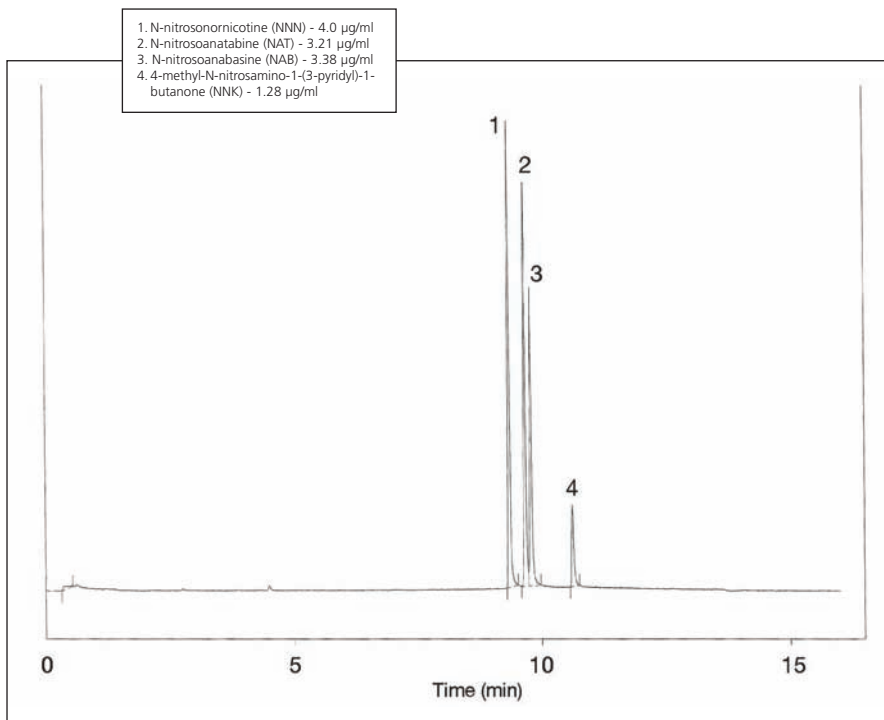


Figure 4. Chromatogram of Tobacco Specific Nitrosamines (TSNAs)

## Food

It has been normal practise for many years to cure meats using salts as a preservative, to enhance flavours and prevent formation of toxic products produced by *Clostridium Botulinum*. Sodium nitrite and sodium chloride are commonly used as the curing agent in meats, however, because the meats contain amines the combination of these chemicals can form nitrosamines. Nitrosopyrrolidine and NDMA are the most common compounds formed. Bacon is the most studied cured meat, due to the relatively high cooking temperatures which helps in the formation of nitrosamines. Addition of ascorbic acid or erythorbic acid reduce the formation of the nitrosamines. Large scale processors of meat are required to add these nitrosamine inhibitors, however, with the current trend to many more small holdings producing their own meat for consumption and sale at local markets, the controls that have been put in place to reduce nitrosamines over the last 30 years could be undermined. Testing labs should be available to check small and large scale productions of cured meats.

The level of nitrosamine formation is also dependent on the cooking method employed. Frying is considered to produce more nitrosamines than grilling and subsequently baking, due to higher temperatures the meat proteins are subjected. Barbeques have also been found to produce a high level of nitrosamines due to the cooking temperature and it is also suspected that phenolic compounds from the smoke can aid in the creation of NDMA and other nitrosamines. This reaction is also thought to be the same for smoked food stuffs such as fish, bacon and cheeses.

## Water

It has been reported that in some waste treatment systems the combination of waste products, shampoos, cleaning products and detergents break down to form compounds, which can later react with other chemicals to form nitrosamines that can re-enter the domestic water supply.

NDMA in domestic water systems are mainly formed by the reaction of organic nitrogen compounds with chloramine. Present in the organic nitrogen compounds are dimethyl amines which are formed from the breakdown of the chemical soup found in waste water treatment plants. For many years the domestic water supplies have been treated with chlorine or more recently chloramine as a disinfectant to prevent bacterial growth. The combination of these chemicals can give rise to significant levels of NDMA.

Even intake through exercise has been observed with occasionally frightening levels of nitrosamines observed in swimming pools. Due to higher amine precursors from urine, sweat and faeces, the increased likelihood of production of NDMA can be observed in pools and hot tubs. Chloramination is a common method of disinfecting water in pools and hot tubs. Chloramine has been demonstrated to combine with dissolved oxygen in aqueous solution to produce NDMA. It has been shown that indoor pools were found to contain higher levels than outdoor pools, with this attributed to UV light as well as better ventilation at the outdoor pools. Although NDMA is widely known to cause bladder cancer through drinking infected water, the effects and ability of NDMA to permeate skin is currently unknown. Levels of NDMA have been found to be up to five hundred times greater in swimming pools, than in normal drinking water.

## Beer and Whiskey

In the malting process barley is first drenched in order to get the grain to germinate and then the grain is subsequently dried in kilns in order to freeze the sugar and the flavour compounds for the brewing process. It was found during the kiln drying process that nitrosamines were being formed in the grain which would be subsequently transferred to the brewed product. Many new techniques are now available during the malting process to minimize the formation of nitrosamines, however, low levels of the carcinogenic compounds still remain. Therefore, all malt used in the brewing process needs to be analysed for its nitrosamine content. As well as the malt, it is important to monitor the end product to ascertain the levels in the consumed liquid. The rise of micro breweries could be a cause for concern, as they do not have the equipment like the big multi-national brewing companies to monitor the nitrosamines. Also, by-products of the malting/brewing process are used for the manufacture of malt extracts and vegetable protein based products and are liable to contain levels of nitrosamines.

## Rubber

Natural rubber is often chemically and heat treated during the vulcanisation process, creating a change in structural properties. During these reactions, a large amount of nitrosamines can be produced. The chemicals often used are based on derivatives of secondary amines e.g. tetramethylthiurame, zinc-diethyldithiocarbamate or N-oxydiethylene benzothiazolylsulfenamide. Many of these chemicals contain a background level of n-nitrosamine contamination. However, the majority of the nitrosamines which occur during the vulcanisation process are when accelerators based on amines come into contact with nitrosating agents.

These nitrosamines can be contained within the rubber and get into the body in a multitude of ways. Particular concern has been shown with respect to baby products. The most common method of transfer into the body is through insertion into the mouth. Therefore baby bottle teats and toys made from natural rubber have shown measureable levels of nitrosamines and now have strict monitoring procedures. The sampling usually involves submerging the rubber in a synthetic saliva solution to mimic a child sucking on the product. The nitrosamines are then extracted into an organic solvent and analysed with GC fitted with a TEA. A similar approach is applied to balloons as they also possess the capability for transfer of the carcinogens to the mouth of the inflater.

Studies have also been performed into the nitrosamine migration from condoms into physiological fluids. Nitrosatable substances were also investigated as both saliva and vaginal mucus could have been a suitable medium for the production of nitrosamines.

## Cosmetics

Raw materials for the production of cosmetics have been found to contain nitrosamine impurities. Both Europe and Canada have banned nitrosamines in any cosmetic product, therefore, these raw materials need to be screened before use. However, formation of secondary nitrosamines can occur from reactions between some proteins and the preservatives, such as diethanolamine or triethanolamine. These compounds are common additives in cosmetics used to adjust pH or act as a wetting agent. It is reported, that one in every 10 products may still contain the compounds that can combine to form nitrosamines. A range of inhibitors can be added to the cosmetics to prevent formation of n-nitroso compounds, but the effect of each is not completely predictable. Therefore a substantial amount of testing for nitrosamines must be carried out within the cosmetics industry.

## Agrochemical

In the manufacture of pesticides and herbicides, secondary amine dinitroanilines are commonly used as precursors. These can contain nitrosamine impurities with surprisingly high levels. Also in the presence of nitrites, another substance commonly employed in the production process, nitrosamines can be produced. In the agrochemical industry, it is often not essential to know the exact profile of nitrosamine species, but a total composition is often reported.

## Gastric Juices

Many vegetables including lettuce, potatoes, beetroot, carrots, spinach and cabbage have various levels of nitrates, dependant on how they were grown, what fertilisers were used and when they were harvested. With these vegetables and meats which contain amines within our diet, nitrosamines can form in the gastric juice of the human stomach. Some scientists claim that the natural vitamins in these foods help prevent the formation of the nitrosamines, but further investigations need to be performed to confirm whether our modern food production methods are transforming humans into manufacturing sites for these carcinogenic compounds.

## Atmospheric Nitrosamines

The inhalation of airborne nitrosamines formed in workplace atmospheres can present a significant risk to workers. The presence of amines and nitrogen oxides with certain levels of humidity and temperature can cause the formation of airborne nitrosamines. In rubber production, as mentioned earlier, vast amounts of NDMA can be vented into the atmosphere, enabling inhalation by humans. In metal working facilities, fluid used as corrosion inhibitors in turning and grinding processes, have been found to be a potential source for nitrosamines. In the leather industry, nitrosamines formed in the tanning process can become airborne in leather dust. Levels of nitrosamines in the air can be monitored by workers wearing an air sampling cartridge such as a Thermosorb-N (Figure 5), which can then be analysed by GC TEA.

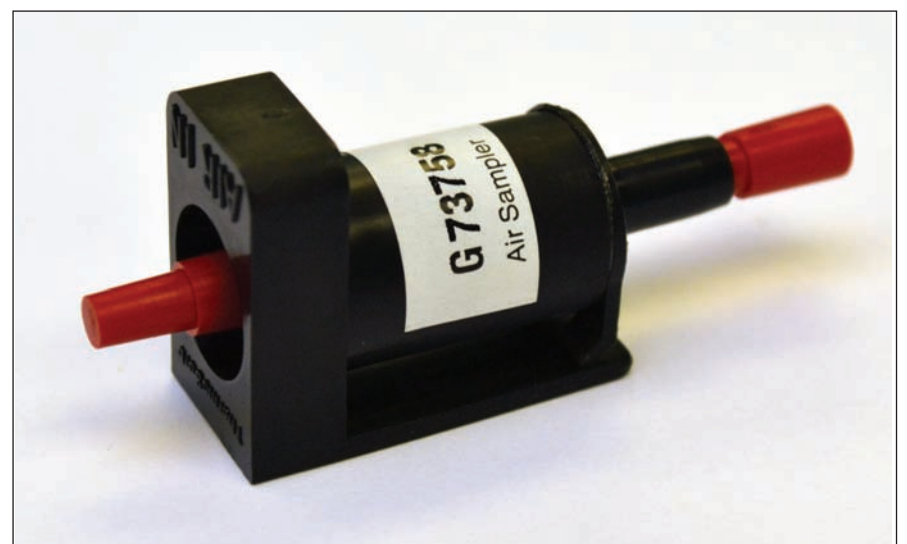


Figure 5. Thermosorb-N Air Sampling Cartridge

A vast amount of NDMA is produced during rocket firing. Commonly the bipropellant fuels consist of 1, 1-dimethylhydrazine and nitrogen tetroxide. As the NDMA produced is airborne a large area can be contaminated in a short space of time due to wind and general dispersion. The nitrosamine can then easily contaminate the water table and surrounding land to alarming levels.

## Analysis of Nitrosamines

### GC TEA Analysis

Components are first separated using a GC, the GC effluent is then introduced into a pyrolyser. In the pyrolyser under vacuum, nitroso containing compounds cleave at the N-N bonds, releasing the Nitrosyl radical. The pyrolyser reaction products then pass through a gas-stream filter which allows only the nitrosyl radicals to pass through.

These nitrosyl radicals then react with ozone under vacuum to produce electronically excited NO<sub>2</sub>. The NO<sub>2</sub> rapidly decays to its ground state emitting light in the near infrared region which is detected by a sensitive photomultiplier. The signal is amplified and displayed on either an integrator or strip chart recorder (Figures 6 & 7).

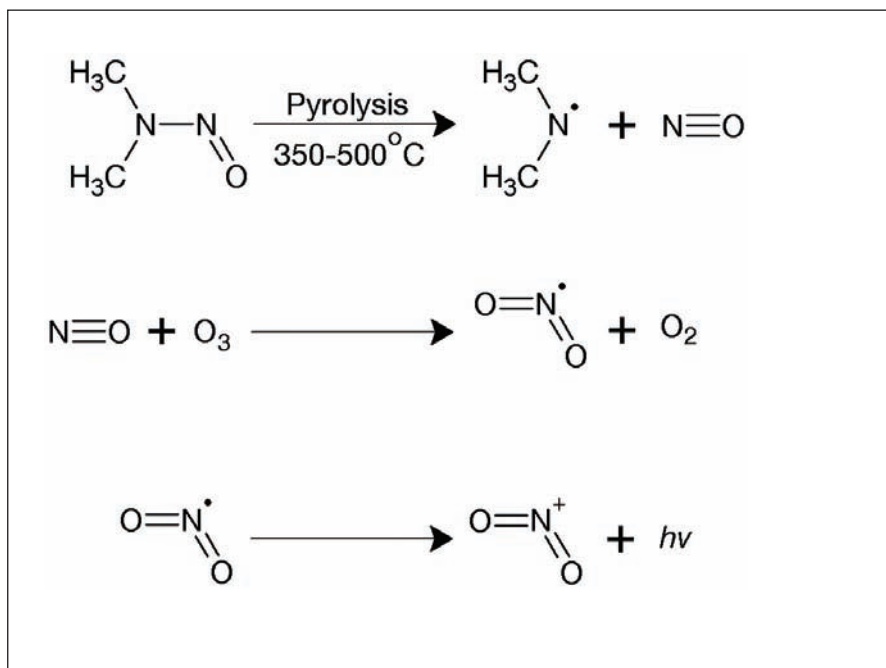


Figure 6. Chemical Process in GC TEA Analysis

### Chemical Stripping TEA Analysis

Instead of using the pyrolyser to heat the nitrosamine to remove the NO group, a chemical reaction can be employed. The alleviation of NO from the nitrosamine is performed by a reflux reaction with hydrobromic acid in ethyl acetate. The nitrosamine sample is injected into the reaction vessel and produces NO, a secondary amine and bromine. The NO is then carried in a gas stream through a cold trap (removing any vapour) to the TEA (Figure 8).

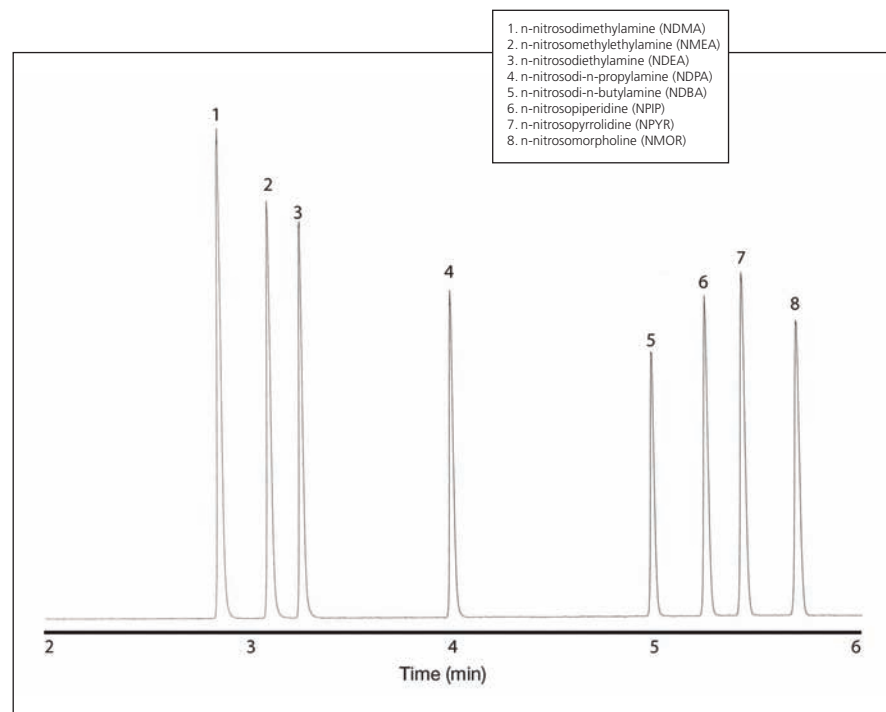


Figure 7. Chromatogram of Volatile Nitrosamines

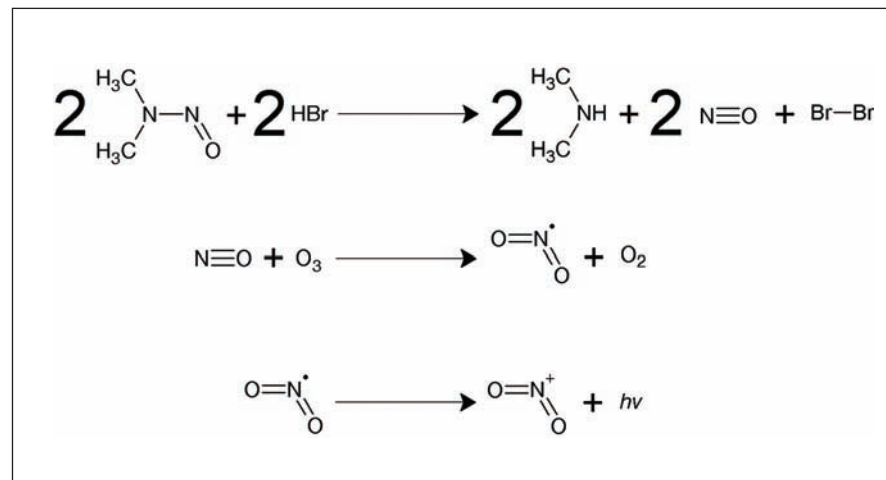


Figure 8. Chemical Process in Chemical Stripping Analysis

## Conclusions

Nitrosamines have a vast research history, indicating their severe carcinogenic effects on human health. For this reason it is imperative to monitor not only the end product for these cancer initiating substances, but also restrict the use of nitrosatable chemicals as precursors.

Thanks to its low limits of detection and selectivity the TEA has a long proven record at being a suitable GC detector for the determination of the volatile and tobacco specific nitrosamines directly, as well as the non-volatile nitrosamines with derivatisation.