Edited Transcript of Workshop*

'LATEST ADVANCES IN CORK-PROCESSING TECHNOLOGY
12TH Australian Wine Industry Technical Conference, Workshop W19
Sunday 25 July, 2004

Chairman, Dr Mark Sefton, Principal Chemist, Australian Wine Research Institute
Convenor: Dr Miguel Cabral, Head of Research and Development, Amorim

Mark Sefton, Chairman (Australian Wine Research Institute)

SEFTON Welcome to today's workshop, 'The latest developments in cork processing technology'. For those of you that don't know, I am Mark Sefton and I am from the Australian Wine Research Institute and I'll be chairing the workshop today. We've got a really busy program, we've got a number of talks but we also have fairly long session for panel discussion so can I please ask that you keep your questions on individual talks to the discussion period when all the speakers will be present and we can talk about either the individual talks or about anything that you wish.

The first session really deals mainly with approaches to dealing with cork taint, and then at the end of that session there will be a panel discussion, a break… Sorry there will be a break during which some wines will be presented. Now some of these wines are wines that have been stored in bottles with corks before and after treatment, which you will hear about today, to see the effect the treatment has on the wine. The white wine in this set-up is perhaps a little past its best, it's designed to be drunk young I am told, but it has been left in the bottle for a long time so that you can see for yourself the effect of this processing technology longer term as well as the shorter term. It is important that these technologies work over more than just a few months, obviously.

In the second session we will deal mainly with cork as a barrier to oxygen and there will also be a second panel discussion in this field. So, I would like to start. Most of the presentations that you will hear today will be by the cork processing industry, and I have been asked to give a very brief overview of just what sort of work is going on here in Australia amongst researchers, what we are doing here as well as what the cork industry is doing. Now this will be very brief, but there will be more details about

---

* For a full transcript of this workshop, contact Clifton Consulting Services by email at clifton@cliftongroup.com.au or Tel: +613 9654 3522.

some of the other things that I will be putting up here on posters put up at the conference and in some of the presentations as well.

There are a number of cork-related issues. The ones that I will be talking about today and which will also feature with other speakers are, obviously, the issue of cork taint; that is very important to many of you here and to the many representing their companies. Something that is a little less often talked about is the issue of flavour scalping or absorption of flavour compounds from wine by closures. I want to say at the outset that the fact that some closures may or may not absorb certain compounds out of wine, doesn't necessarily mean that it makes the wine better or worse. It depends on what the wine is and what is in the wine in the first place. We've had examples in the past where closures have actually removed taint compounds from wine and made an unsaleable product a saleable product after a period of a couple of years. So, it's not a simple matter of scalping. It's something that takes flavour out of the wine and therefore makes it worse. And the other issue, which is subject to quite a bit of research at the moment here, in Australia, is the issue of premature oxidation in bottle and I will talk about that in a few minutes.

OK. There will be a strong focus today, obviously, on TCA (tri-chloroanisole), which is one of a group of chloroanisoles, only two that really effect wine quality—that's tri-choloroanisole and tetra-chloroanisole and really only TCA is associated with cork closures. Wherever we have seen tetra-chloroanisole in wines it usually comes from other sources. It's a very occasional, infrequent wine problem that usually comes from contaminated wood or something similar.

In past years we have had a lot of research efforts in TCA looking at the interaction of cork in the bottle and the distribution of TCA between wine and corks; but more recently we've had a focus on other possible sources or origins of cork taint. There are a number of compounds that have been implicated in cork taint and they are only implicated. These are the things that have been isolated from cork; whether they are really a problem or not is something that I feel has yet to be demonstrated. For example, guaiacol has been mentioned as a possible source of cork taint, but guaiacol is also a natural wine component, especially in red varieties, and it is a component of oak wood, so guaiacol can be present in quite significant concentration in barrel-aged wines.

More recently we have seen abnormally high amounts of guaiacol in wines produced from fruit that have been grown in areas which have had bush fires near by, so, I don't think that cork is a particularly serious source of guaiacol in wine. These compounds here—methyl-isoborneol, geosmin—they are associated with earthy taints in other food products. They have occasionally been found in corks but no one has ever really demonstrated

that they are a serious cause of cork taint. And likewise these compounds have more of a mushroom character, have been isolated from cork, and I've yet to see any real evidence that they are a serious problem. Some of you may have different opinions and may have seen some of these taints, so that is something that we can discuss.

Some of you sitting here may have been at an ASVO seminar ten years ago, when Blair Duncan gave a presentation on the sort of taints that were encountered by Southcorp Wines during their cork screening. They classified these taints, and the most common according to their sensory panel was not surprisingly TCA. But there was a second taint, the second most prominent taint that they came across was something that they call fungal must and fungal most often seemed to co-occur with TCA. Some cork soaks would have elements of both of these, and they found that some of their panelists found it hard to tell these two things apart. They were not all that different to a lot of panelists.

We had a look at some of these cork extracts at least ten years ago, but we weren't able to identify what was responsible for this, but what we could show was that this fungal must character wasn't anything new to anything that had previously been isolated from cork. It was some unknown compound that no one knew anything about. Well, in the last few months, really due to the terrier-like efforts of my colleagues—one of whom I thought would be here, but she is out pouring the next workshop I think, Dimmy Capone and Bob Simpson—we finally isolated and identified the compound responsible for fungal must. It's not published yet, but it should be coming out shortly.

It's 2-methoxy-3,5-dimethyl pyrazine. A lot of you have heard of methoxypyrazines, they're associated with Sauvignon Blanc and Cabernet Sauvignon fruit. This is structurally similar in that it has the pyrazine ring, the methoxy group and the methoxypyrazine that you get from grapes have a substituent here as this does. This compound also has a substituent over it in this position, but physically and chemically you would expect them to behave similarly to other methoxypyrazines, and now that we have identified this, we are in a better position to know how to deal with it in corks, although we are yet to determine just how frequently it occurs.

So, I'm not going to say too much more about this. There is a poster up, it is poster 211, if you're interested, which Dimmy Capone will be presenting. And I will also be talking about this in my presentation on Wednesday afternoon a little further.

We have also been looking at the issue of absorption of wine compounds, flavour compounds by closures, so-called flavour scalping, which is a known phenomenon in the packaging industry generally. We've looked at

a number of different closures, screw caps obviously, two types of natural bark cork, a technical cork closure, and seven different synthetic closures and we've analysed wines after two years of storage and we'll be looking at five year storage period fairly soon, and, comparing these wines with controls stored in all-glass containers, so we can see what difference the closure makes.

.

I am just going to summarise briefly because again I will be talking about this in more detail on Wednesday afternoon. Not surprising after two years that the concentration of some compounds changes; you would expect that wine flavour changes, so that not surprising. But, not all things change in concentration over that period, some things are completely stable. Some of these changes are only due to chemical reactions. They are nothing to do with the closure, nothing to do with whether the air is getting in or not, they are just natural changes that are happening to wine, regardless of how it's sealed. But there are some changes that are attributed to absorption by the closure, not due to oxidation, but to the closure's actually drawing them out of the wine. And the other general statement I can make is that with the screw caps there is no absorption of any compound of any kind at all observed. Now again, I will elaborate on this again in a few days time or we can talk about it in the discussion if you wish.

Here's just an example of some fermentation esters. This is as a percentage of something we see in a control line with no closure. So you can see that for this particular ethyl ester, the natural and the technical closure have no effect. There is a slight absorption by the least absorptive synthetic closure, a little bit more than the most absorptive. When you get to the longer chain, esters absorption becomes more significant particularly for the synthetic closures. Now there are some compounds that are more substantially absorbed than these. Others, probably the majority of others, aren't absorbed at all. But this gentle trend of the synthetic closures absorbing most and the technical and the natural closures absorbing the least and Stelvin not absorbing anything, was general whenever we saw absorption. It's not that some closures absorb some compounds more than other compounds and it's a different closure. When you get absorption it is always the same closures that have the greatest absorptive capacity.

The third area which I personally haven't had much effort in, but my colleague here in the front row George Skouroumounis, has been working in for many years now, and Liz Waters and other colleagues at the AWRI, is looking at development of oxidative characters in wines in bottle and this can be measured in a number of ways. You can measure simple absorbance on A420 or you can measure sulphur dioxide consumption. And the two are highly related as you can see in this set of wines, which

has suffered from random bottle oxidation and this is something that Liz Waters and others did quite a few years ago.

Thanks to this invention by George here, which was presented by poster in the last conference, it is now possible to measure absorbance in wines without opening a bottle with this simple modification he has here. And although this shows a clear glass bottle, you can measure absorbance in any colour at all, any coloured glass used in the Australian wine market at the moment, you can measure absorbance with this instrument. And that has enabled several companies who have problems with premature random bottle oxidation to screen their stock, screen out the ones, which have seemed to have suffered more oxidation and keep aside those that seem to be performing better.

So, that has also enabled a lot of research and looking at how wine is developing in bottle, and I'll just rip through this quickly. This is showing a series of wines with and without of ascorbic acid, and you can see even the wine under the screw cap closure still continues to get darker. That's the one without ascorbic acid, so even though there is no air getting in, the absorbance still increases over time. When you have other types of closures the absorbance also increases but at a greater rate, particularly again for the synthetic closures. That suggests that perhaps this darkening of wine is a process that doesn't necessarily require oxygen and you can see that also in this slide. Now this was a wine that was opened after a few months and divided up into smaller lots and a fixed amount of oxygen was added at this point, and then these wines were stored under a nitrogen atmosphere in what we call an anaerobic fume hood, so there is no way oxygen can get in. There is no oxygen around to get in in the first place, and you can see a number of things.

First of all the wine with no added oxygen and no possibility for any more to get in still continues to get darker after this period of time, but, the wines with oxygen added still continue to get darker and they get darker slightly faster. And you can see that you can still discriminate from the lowest amount of oxygen from the highest by measuring absorbance at A420. But some of this increase in absorbance is what we call an anaerobic process, that is, it doesn't need oxygen. So, wines don't need oxygen to get darker, but if they get oxygen they will get darker faster. So looking at the differences within a set of wines can give you an idea of which ones are suffering more than others.

The other thing to notice from this is even after two or three months, the oxygen hasn't had much of an effect on colour. So, if you are starting to see difference in absorbance in wines after a long period of time, it may simply reflect, not something that is going on right there and then, it could reflect something that happened a long time ago, even during bottling or

perhaps even before. So, you just need to be aware of this. That doesn't mean that it's not due to closures, but you need to keep these other possibilities in mind as well, because the effect of oxygen on colour can be a very slow process.

I said earlier that absorbance at 420 is used as an indicator of oxidation of wines. This (slide) shows the relationship between absorbance, A420 and a panel's score of the oxidised aroma of wines. But, this looks a bit like my archery target on a bad day. There doesn't seem to be much of a strong correlation there. This is a line with and without ascorbic acid stored under different closures, which had different degrees of oxidation taking place. When you divide these lines up into those with ascorbic acid and those without then you can see the relationship, and the problem here is you cannot compare wines with and without ascorbic acid using absorbance. They would behave as if they were different wines.

So A420 is not a good way alone of seeing what the effect of ascorbic acid is on wine, because these wines with ascorbic added, especially this group here, they have the highest absorbance but they are not seen as oxidised on the nose as this set of wines here without ascorbic acid. So you need to be careful when using this measurement to compare wines. You can only compare wines within a set. Just as you wouldn't compare a Riesling with a Chardonnay by looking at their color you would only look at different bottles of the same Riesling in order to work out which one is more oxidised, you can't compare that without ascorbic acid.

This (slide) just shows the different closures of the wines, those wines were stored under. Those under synthetic closure, which had the highest absorbance, also had the highest oxidised score, particularly those with ascorbic acid added, in this particular case. Please be careful, it is very easy to generalise from case studies like this and say therefore we should always use ascorbic acid. You can do another case study sometimes and you'll see something slightly different. You need to see what works with your own wines in your own storage conditions before perhaps making changes to the way you might do things.

The wines under natural closures shows less oxidised character and not surprising those under Stelvin showed the least oxidised character. Although I would have to say that these wines here had a slightly reduced character to some assessors, so again I am not saying anything about which wines were better, and which ones are worse. I think that George and his colleagues are presenting a workshop in which you'll have the opportunity to taste some of these wines, if you are going, and people who attend those workshops can judge for themselves.

And a final issue that is being investigated at the moment is whereabouts exactly is the air getting through? Is it getting through the centre, or the body of the cork when air goes into a wine, or is it actually something more to do with the interface? And you can look at this by playing around with Araldite—Araldite is a very good barrier to oxygen—and again there are a set of experimental wines being looked at at the moment, some which aren't sealed, some that are completely sealed, some of which where this part here is left open (and this was an earlier prototype—not very well sealed as you can tell by the shape of that blob), and there are others where only the central part is sealed and only this interface here is left exposed to the air. And that tells us whether it is the matrix of the cork itself that is likely to be a problem, or should we perhaps be looking more carefully at bottlenecks specification and lubricants and seals for corks. Is that perhaps more of an issue than we realise?

All right, that is a very brief and thumbnail sketch of what's been going on and what's likely to go on in the future mainly at our Institute. The remaining speakers will talk about what they've been doing to try and improve closure quality. So our next speaker is Miguel Cabral.

Miguel has directed the research for Amorim. He has a PhD in microbiology, which he got from the University of Strathclyde in Glasgow and a Bachelor of Pharmacy from the University of Porto. He has been a professor in parasitology in the Pharmacy Faculty of the University of Porto since 1994 and he still holds that position whilst he is working for Amorim. Before joining Amorim, he was head of the Quality Control Laboratory at the Vinhos Verdes Institute. He is the author of over thirty scientific papers in parasitology, microbiology and oenology. Thank you Miguel.

Miguel Cabral (Head of Research and Development, Amorim)
'Alternative approaches to defeating TCA'

CABRAL Good morning everybody. I am going in my first talk to present the alternative approaches to defeating TCA that the cork industry has been doing in recent years. These kinds of approaches I will divide into two main areas. The first, which I call industry-level, are initiatives that are happening more or less in all major companies at the same time. In the second part I will talk about the initiatives all the individual companies are taking in order to defeat the problem of TCA and I will divide them into several different groups that I will explain in a moment.

Starting at the industry level… in recent years after a research project financed by the European Union, called the Quercus Project, we have developed a code of good practice to create standards for the industry, for

each practice in the industry. This code of good practice is now applied by cork companies. And the companies that want to be certified under the code have independent audits made by Bureau of Veritas.

This has been applied already in the industry and we have, as you can see here, a significant number of companies already certified in Portugal, in Spain, in France and also Italy, Germany and Morocco. So, this industry action, which is guided by the C.E Liege, has significant implementation within the industry at this stage.

The second big change that has been also applied across the industry in general is the change in quality control. We are moving from sensory to chemical analyses in quality control following a study by ETS Laboratories. That study was commissioned by the Cork Quality Council in the United States, which is a group of five cork companies, now six cork companies. And they asked ETS Laboratories to do a study to try to have a much more objective way to quantify TCA. That has been done and now (the method) is applied through the majority of the cork companies, and my colleague Stefan Dahl will speak in detail about that later on in this workshop.

Now concerning individual company initiatives, we start with TCA extraction—initiatives to extract as much TCA as we can from cork. Then individual initiatives in terms of microbiological disinfection, because we know that TCA is formed after the transformation of some precursors, mainly tri-chlorophenols, by the action of fungus. So we have several different initiatives to reduce the microbiological burden of cork stoppers, in order to avoid that transformation.

Also functional barriers, there are to our knowledge two initiatives to establish a barrier to avoid the migration of TCA into wine. And finally enzyme treatment that my colleague will talk about, which is Suberase treatment.

Let's go first of all to TCA extraction, and we all know that in the last three to four years, probably a little bit more, the cork industry has changed significantly the first industrial process, which is the boiling of cork. As you know, we boil cork because we need to expand cork in order to have enough thickness to punch the corks. The old way of boiling was a very static way and the new boiling is a much more dynamic way as I am going to show you in the next diagram.

Here we can see the old way of boiling, the new way of boiling. And in the old way of boiling we had a big tank, used normally for around five pallets of wood. Then we compressed those pallets during one hour of boiling at 96 degrees, 98 degrees, in a static process. Then when the

process was finished we released the compression and so the cork, like a sponge, sucked water from the tank, so it came out with a high humidity level.

In the new system, and here is a good example of a new boiling system for cork, we have a much smaller tank first of all. Secondly, the cork in the tank is not compressed, so each cork plank is floating more freely in the water and so the water can pass through the planks more easily. And the water is continuously passed through the system to remove volatile compounds and so avoid cross-contamination. When we extract some TCA or other volatiles from one plank we try to avoid having that TCA passing to another cork plank. We do that by what we call a volatile continues trap (CONVEX). During the boiling process, water is passed from the tank, through the filter, then a heat exchange to maintain the temperature, and then it comes into the trap. Here water is opened in a curtain to increase the contact surface. Then we blow air into that curtain and the volatiles come out. Don't forget the water is at around 96 degrees, so it's much easier to extract volatiles with that system at that temperature.

The final result is that in the old system we get cork with a moisture content of around 40 per cent. So we need to wait some time to get a moisture content of around 12 per cent, which is the ideal moisture content for punching corks, and so during this period we have a high possibility of fungus growth. Conversely, in the new system we have much lower moisture content and so we need much lower time to get the same humidity to punch corks. Having much lower time we will have a low possibility of mould growth and so, if there are precursors for TCA present, we have low possibility of the formation of TCA.

So, this is what has been implemented in several different factories, but we have other systems to extract TCA that have been shown to the market already. One good example is the Ultracork, which is an English company that developed a system where they extract TCA with hexane in the presence of ultrasound. They wash corks with hexane in the presence of ultrasound. TCA will be dissolved in hexane and so extracted from cork. Then they apply a surface treatment of silicone, which they claim to be a barrier for TCA. So, they reduce significantly the migration of the remaining TCA. So, this is Ultracork, and I have to tell you that I don't know if it is already in industrial phase or not.

In other approaches, as some of you have probably already heard, there is controlled steam distillation, the ROSA system, and I will present my results for ROSA in the next presentation from Amorim. And the presentation after mine is from Sabaté, on the super-critical fluid extraction using CO_2. Jean-Marie Aracil will present the results to tell you about that approach.

Another approach that has already been presented to the market is the Delfin process. Delfin is direct environmental focus innovation. It's based on microwave microbiological disinfection that is going to significantly reduce or even sterilise corks or reduce the microbiological burden in a significant way. But they also say that it reduces TCA because they can extract TCA with this microwave system. This has been developed by a Portuguese company, Juvenal, in conjunction with a German researcher.

Another system to use microbiological disinfection has been presented by Ion Med, which is a Spanish company. Ion Med uses ionisation by beta radiation to reduce the microbiological burden of cork stoppers. So again it is the same principal, it is a way of trying to sterilise corks or to reduce as much as they can the microbiological burden in order to avoid the formation of TCA. Again, they claim in this case that they can get some reduction in TCA but I don't know what the results are. I have not seen the results.

Another action in terms of microbiological disinfection is the gamma radiation ionisation that you have already here in Australia. Four years ago I visited two disinfection units of gamma radiation. Now a Portuguese company C.H.I.P. is using that in order to reduce TCA from corks. And also several different companies are using ozone, in gas phase or dissolved in water, and the objective is always the same, to reduce the microbiological burden. Actually there are some companies that claim that ozone can directly reduce the TCA in corks.

Now to functional barriers. There are two cases of functional barriers presented in the market. The first one is the one presented by Cortex, which is a French company, and it is called Le Preserveur. It is a band of silicon, a thick band of silicone that in contact with the corks will act as a physical barrier to the migration of TCA. And so they claim very good results with this kind of barrier. They are applied to natural corks but also to champagne corks.

Another functional barrier is presented by ProCork, an Australian company. They presented an idea that is more or less the same. It is a barrier to avoid the migration of TCA, but in this case there is a thin membrane made with several different layers. And with this thin membrane, they say, the passage of TCA into the wine is avoided. Also it seems that this membrane slows the passage of oxygen through cork and reduces cork moisture. So, in terms of functional barriers, this is what we have.

Finally we have enzyme treatment. We are going to hear a presentation about Suberase so I hope I am not going to say the wrong thing. Basically,

Suberase is an enzyme able to polymerise phenols and so avoid the formation of TCA. This is because the phenols are mainly tri-chlorophenol, which is the main precursor for TCA, and it's not going to be available to be used by fungus to produce TCA. This (Suberase) is the Novozyme enzyme treatment.

So these are the different company initiatives. These individual initiatives from all the different companies show that the cork industry is clearly aware of the TCA problem and is committed to solving the problem. And of course all these initiatives clearly tell us that we have made real progress in this cork taint problem and that TCA as a risk factor to wine is being reduced.

So this is the first part of what I want to tell you concerning the general initiatives by the cork industry for defeating TCA.

CABRAL **'The ROSA process for TCA extraction'**

But now I will talk in a little more detail about the initiative our company, Amorim, has made concerning the TCA.

Our approach to TCA is not just a cure, but it is also an approach of prevention. Prevention is very important, because by having lower amounts of TCA it is much easier to cure, to remove the TCA from those corks.

We have several different preventive measures. First raw materials. We deal in a different way with raw materials: less and less we store the cork planks in the forest, and more and more in the factory. We remove the lower part of the cork bark that is in contact with the soil, because if it has the possibility of contamination it has a high level of contamination and so we remove that bit before boiling to avoid cross contamination.

Second, cleaning. We have the new boiling system, more or less the system that I have already explained to you. We have INOS II in order to wash cork discs and also bar top corks. Third, we have ozone in order to reduce microbiological burden. But apart from all this we have chemical analysis and we are doing more and more chemical analysis to get away from sensory analysis and to use a much more objective way of quality control. Stefan will talk a little bit more about that and I will talk about this curative process, which is called ROSA.

ROSA means Rate of Optimal Steam Application. Basically the idea came from the Merck Index. We were brainstorming, thinking about what we can do to defeat the problem, and Stefan Dahl was the guy who says, let's

see what's happening in the Merck Index, the book that talks about all the volatile compounds. And actually the Merck Index says not too much of TCA but in the middle it says that TCA is volatile in steam. And Stefan says, why not try steam application, let's see if it works or not. And so that led to a big project, a three-year development project, where we started at the lab scale, then we went into prototype, semi-industrial scale. Actually we had the first prototype, then we had to build a second prototype, and I will tell you why in a moment. Then we went to an industrial pilot, we validated the system and now we are in full industrial production for cork granules and in industrial routine for cork discs and stoppers.

Let me explain first of all, how the system works. It is actually a very simple system. This is prototype one. Cork granules come into the system from this hopper, then they are passed through a tube by this auger and in between we inject steam through the tube. We show here just two injectors but actually we have more than that. And so the steam is extracted there and the granules are recovered here. Very simple system as you can see.

This system works very well for granules and also for corks and stoppers, but this way of transporting corks and stoppers (via an auger) was damaging the edges of the corks. So we had to create a second prototype, not a continuous system but a batch system (using a tumbler). The steam is injected and we have several exits where the steam is extracted. After half an hour we take out the cork discs and stoppers from the tumbler.

Very simple as you can see but there are some details which are very important in this system. And the first and very important detail is the temperature of the steam. The second one is the flow of the steam and the third and probably the most important is the extraction of the steam. It's a very important detail, the extraction of the steam, because if we don't extract in a proper way we have condensation of the steam and so re-contamination of the granules that are already there.

OK, so let's show you the results from the (validation) studies for granules. I'm not going to show you results from the lab scale and the prototypes because we are already in industrial scale. So, here are some internal validations for granules on different days. Each of these groups of two bars corresponds to a different day, where we have, before ROSA a significant amount of TCA. Of course these are granules we detected as contaminated and we use them for our validation. This is because we want to start with highly contaminated granules in order to see the difference. We made the validation, in two cases with eight samples and then thirty samples before and after ROSA, and in all other cases ten samples before ROSA and ten samples after ROSA.

You can see that the differences are all above 70%, having the worst case of 70.4% and the best case where we have more than 93% reduction. So very good reductions, large enough to proceed further. And we decided to go to independent validation. We started by asking the Geisenheim Research Institute in Germany to validate the system. Rainer Jung was with us one week to validate the system. From a contaminated lot of granules, he took 50 samples before ROSA and 50 samples of granules after ROSA and he got a very good average reduction of 75.1%. So, very good reduction, similar to the ones done by us.

The second validation was the Campden and Chorleywood Food Research Association from the UK. Nick Byrd was with us for one week to do exactly the same. So we did 50 samples before ROSA and 50 samples after ROSA again from a batch of contaminated lots of granules. Look at the reductions he obtained, an 80% reduction. So, again similar results to the ones done by us.

Finally the Australian Wine Research Institute, Mark Sefton, was with us for one week, making the same thing but in a different way. Mark asked us to mix two bales of contaminated granules. We took six samples before passing through ROSA, we passed two bales through ROSA and then we took the other six samples after ROSA. The six samples before and after were called units, and we made eight units. So at the end we had around 48 samples before and 48 samples after ROSA. The results from Units 2 to Unit 8 were very consistent and very good, a reduction of around 72.5%, but in Unit 1 the results were not so good. But including this result for Unit 1 we have a 69.3% reduction. It is important to say that, we did a parallel sampling of these units, and the results for Unit 1 were very similar to the others. So there is probably some problem here.

OK, so we can say here concerning cork granules that the reductions were very good and very consistent across the independent validations. We have 75.1%, 80%, 72.5% or 69.3% if we include Unit 1, all very consistent results and very good reductions, all from very highly contaminated granules.

But it's important to note that steam distillation is not a system specific to TCA and we found similar reductions for other volatile compounds such as guiaicol and geosmin.

We analysed 10 samples before and after ROSA to measure the presence of these compounds. And as we were expecting, we got very good reductions, similar reductions as the ones obtained for TCA.

Here is a picture of an industrial unit of ROSA, we have more than seven now spread through our factories.

But what you want actually is to see how this works in terms of wine, because that is the most important thing for you. And these results are obtained in a bottling experiment using corks treated at the prototype scale. We divided a contaminated batch into two parts.

One part, we passed through ROSA and produced agglomerate cork stoppers and the other part we didn't pass through ROSA and produced agglomerate cork stoppers, and then we used the stoppers to seal a red wine. We were checking for TCA after 1, 3, 6, 12 and 26 months after bottling. The same wine is on the table at the end of the room for you to sample. They now have 36 months in the bottle, so ten more months than here in this slide. The untreated corks have very interesting results in the bottle because you can see the significant levels of TCA we have in the wine. Don't forget that the granules were very highly contaminated. Conversely, the wines after ROSA have a very low amount of TCA as you can see and the great amount of results were less than 2ng/L, which is our quantification limit (on the graph they are shown as 2ng/L but actually they were less than that).

So, we were very happy with the results. This was in a prototype scale so we decided to repeat this similar experiment but after treating the granules in industrial scale. The results shown on the slide are after eleven months and we also have some here for you to taste, which is after 19 months. This is a white wine. (Actually, the wine is not fantastic at all but the objective was not to taste the wine, but to see the TCA before and after.) I don't yet know the result of TCA after 19 months, but after 11 months you can see the difference. We have 25 bottles after 4 months and 25 after 11 month, and with ROSA you have very good results. As you see, the majority of samples are less than 2 ng/L, so clean.

Now let's see what's going on with cork discs and cork stoppers. As I told you already, after the prototype scale, we needed to build the second prototype. That second prototype worked much better and so we moved to industrial scale and we have made the validation, and we are already in industrial routine for contaminated batches.

So, here is a picture of the industrial pilot, which is a tumbler.

Here are the results of our internal validations. Good reductions in general, very good reductions, but in some cases as you can see here, or here, still a problem. This was with the first ROSA prototype, and I don't know exactly why this happens, but for some individual discs and corks the reduction was not so good. But in spite of that fact we decided to go to industrial production and we have got very good reductions as you can see.

In this case we still had a result above 4 ng/L, so we decided to repeat the ROSA treatment for these corks at industrial scale. We were worried about getting higher deformation of the corks, but actually we didn't get higher deformation, but we did get a higher reduction of TCA. Actually this was different from the result we obtained in the lab scale. When we did the lab scale we didn't get that increase in reduction, but in industrial production we have got better reductions. So, we decided to validate the system independently, as we did for cork granules, and we asked Pascal Chatonnet from Excel Laboratories to make the validation of the system.

He was with us for one week and he took 25 different contaminated cork discs. Here are the individual results for the 25 discs before and after ROSA, and you can see very good and consistent reductions. Pascal Chatonnet has made similar validations for cork stoppers, with 25 individual contaminated cork stoppers. They were tested before ROSA and then they passed through ROSA, and they were tested again individually. And again we have very good reductions.

This next slide shows a bottling experiment with cork stoppers. It was done with corks from a batch of untreated cork stoppers and with corks from the same batch that were treated. Unfortunately the batch wasn't highly contaminated to start with, so after one month we have just these two (untreated) corks that were problematic, and after 12 months just a few (untreated) corks were problematic. With ROSA-treated corks we have much better results, but the difference is not so significant because the original batch was not so consistently contaminated as we had thought.

In terms of where we are with the application of ROSA… we are in commercial production for Neutrocork and Twin Top. All our granules for these corks are ROSA-treated. We are refining the technique for granules used for champagne corks, because the density of the granules after the treatment was a problem for champagne corks. We think that in one month, not more than that, we will have the solution, because it is a question of drying after ROSA. If we dry at a lower temperature than we do now, probably we will get much less increase in density. Finally, we are using ROSA for cork discs and cork stoppers, just for contaminated lots at the moment. The uncontaminated lots that are passed by quality control continue in production, the contaminated lots we pass through ROSA.

We will make an evaluation after one year, based on the results obtained in production and also the impact on the visual grade. Based on the balance of the two results, we will decide whether to apply ROSA to all cork discs and stoppers or whether we continue the system that we have now, which is to treat only contaminated lots.

So, this what I want to say concerning the ROSA process, where we are nowadays concerning the treatment against TCA and other off-flavour compounds. Thank you very much.

SEFTON Thank you Miguel.

Our next speaker is Jean-Marie Aracil. He is the Director of Research and Development of Oeneo Closure Division, and has a PhD in Chemistry from the University of Toulouse. He worked for several years in an analytical centre before he joined Sabaté in 1992. He is the author of ten scientific papers in the fields of chemistry and in the character and use of cork as a wine closure. Welcome Jean-Marie.

<Presentation by Jean-Marie Aracil, Director of Research and Development, Oeneo Closure Division not included in this transcript>

SEFTON: We have some wines up the back for you to assess. They are for aroma assessment only they are not for tasting, there are not enough glasses for you to each have your own individual glass. There are two sets of wines. One set are presented by Amorim, which are wines bottled with closures both with and without ROSA treatment. And the others are a set of local wines which have been spiked. Some are spiked and not spiked with TCA, so you can see for yourself what the different concentrations of TCA are like. And there are some sheets in your handouts, these ones here for you to make your own notes on the second set of wines, because Stefan Dahl will refer to these in his presentation. So if you'd like to make your own notes.

Following the assessment of these wines I hope we have some coffees, we've got ten minutes set aside for this break, although we do have some time up our sleaves so I am going to extend that to 15 so that we've got time for a coffee and to look at these wines as well. If you could please be back here at 5 to 10 for the next session, thank-you very much.

BREAK

SEFTON: Our next speaker is Dr Filomena Pettolino. Filomena has a PHD in Biochemistry from LaTrobe University. She has worked for the CRC for Bio-products on commercial related problems in industry based around carbohydrate chemistry. She is currently working on projects with the AWRI and with CUB. Filomena will be talking about Suberase. She is talking on behalf of Novozyme, she is not an employee of Novozyme. So thank you Filomena.

<Presentation by Filomena Pettolino, LaTrobe University, not included in this transcript>

SEFTON Stefan Dahl is our next speaker. He has a PhD in Chemistry and Food Sciences from the University of Gotemburg. He is a Senior Chemist in the Research and Development department at Amorim and before he joined Amorim, which was in 1999, he was a lecturer in Analytical Instrumental Methods in the Biotechnology School at the Catholic University in Porto. He is author or 14 scientific papers in microbiology, biotechnology and chemistry and he has also been a project supervisor at a research institute for biotechnology in Gotemburg in Sweden. Stefan is going to talk to us a little bit about the use GC analysis in cork quality control

Stefan Dahl (Senior Chemist, Amorim)
'The Use of Chemical Analysis for Quality Control'

DAHL Thank-you Mark. Good morning ladies and gentlemen. First of all I want to apologise for reading quite a lot of notes because my English is not so very strong to make it freely. I am going to talk to you about using chemical analysis to help cork producers to supply cleaner corks to the wine industry.

Traditionally sensory analysis was the main tool for detecting TCA and other undesirable compounds in cork. Unfortunately as you know sensory analysis is very subjective. People's sensitivity towards TCA can vary enormously, even for trained tasters. In 1988, members of the Cork Quality Council in the US, which included Amorim as well, asked ETS Laboratories in California to develop a consistent, reliable and efficient tool to replace sensory analysis for quality control in the cork industry. This standardised chemical analysis procedure, developed by ETS was first introduced at Amorim in 2001 and has now almost completely replaced sensory analysis.

To give you an example of the reliability of sensory analysis, this chart compares the judgements of an expert panel of tasters with the results of chemical analysis. The analysis was made using 10% ethanol solution of wine and the 10% ethanol solution even is a less complex medium than wine. As you can see there is a great inconsistency. Sometimes, the panel talks about slightly earthy character in the wine and you have as much as 14.8 nanogram per litre TCA detected chemically, and on the other side around they say moderate TCA and we found much lower concentrations of TCA.

We will probably find the same variation in the results of the sensory analysis you did during the break. I hope you have done it. You tasted a

Riesling and a Chardonnay and they had different spiked concentrations of TCA, and you were asked to note down your assessment and here are the actual TCA levels. Wine A was spiked with 2 ng/L. Wine A was the Riesling. I hope the wine was a good wine and no TCA from the beginning. It was an Australian wine so I think there was no TCA before. Wine B is the Chardonnay and had these values. Nothing, 2 ng/L. We actually chose 2ng/L because that is the level that many people, or many specialists claim that TCA can be detected. As you know people's ability to detect TCA depends on wine style. You may have found that it is easier to detect TCA in a delicate Riesling like the Wine B than in a more full-bodied Chardonnay. As you can see in this slide, Pascal Chatonnet of Excel Laboratories in France found that a panel of expert testers could detect TCA at lower levels in water than in sparkling wines and again, lower levels in sparkling wines than in still wines like the ones you have tasted.

So, people's ability to sense TCA depends strongly on the sensory complexity of the medium. And that affects the requirements for the quality control. What the cork industry needed was an efficient and accurate quality control system that could reliably say whether to accept or reject the batch of corks. The sophisticated methodology developed for the cork industry by ETS, combines gas chromatography, and mass spectrometry with solid phase micro extraction. You will see this thing working a little bit later.

I will explain to you how it works and I have taken this little video, which was taken in our laboratory in Porto.

We start our analysis by taking samples of 50 corks per 10,000 corks as a representative sample. We then prepare a 10% ethanol solution, that is what the lady doing, and then you see she shakes it, and we fill the jar up to the brim. We do this to avoid the loss of TCA through headspace, it must be up to the roof. We leave now the corks to soak for 24 hours in room temperature. Overnight. Now we look a little bit at the preparation of the samples, the tubes. To prepare the samples we add salt, you see, which will be used to force volatiles from the liquid to the gas phase, we call this salting out effect. We then add 10 millilitre of the salt solution that we prepared during the night, in the vials. And now we include an internal standard, which is a known amount of TCA, to check that our analytical results are correct and everything functions. You see she shakes the bottles; that is to promote the salting out effect. The samples are transferred to a tray and now the SPME fibre is injected, you see it here. Now that is the point where the TCA and other molecules present in the head \space are absorbed, then the fibre it is injected in the GC injector and are thermally desorbed.

The chromatograph then separates the chemical components from each other and a detector plots them on a recording chart. Each compound has a characteristic peak, and the area of the peak is the measure of the relative concentration of the compound. You can see here this chart plots the results of several different samples, with several different levels of TCA. And as you can see this is actually an outcome of 1.2ng/l and you have still quite a distinctive peak, an integratable peak. This one was 4.4, this one was 6.7 and the big one, the much bigger one is 14.3 ng/L.

That is a picture of our laboratory.

So Amorim has six of these machines and each is analysing around 75 samples per day and that is a total of, you can see here, that makes more than 8000 samples per month. We can accurately measure TCA levels as low as 1 ng/L, and detect its presence already at 0.5 ng/L. So, you see its quantification is a little bit more tricky.

Because we want to use the chemical analysis as a decision making tool, we must be confident that the analysis and the results are robust. Our methods are validated according to parameters of ISO standard.

That is a control chart that we are establishing and here you see, a control chart of almost a year. In each of the two runs per day, we include control samples for 3 and 6ng/L and we analyse these control samples to check the day's results, the outcome of the day. And this blue line in the middle represents the theoretical value of 3.0 ng/L. I show you just the control chart for 3ng/L. The pink dots, around that line, the spread values, are the daily results of that control sample. If they fall outside of these green lines, max or minimum, which are actually two times the standard deviation, we have to repeat all the samples of that day.

So, you see if our results are very bad or the machine is bad, we have to look for the error, where it went wrong and repeat everything. That is the statistical line, which is the normal alarm concentration, which is three times the standard deviations, but we want to be more sure, actually, so we take this line (2 standard deviations) as an alarm.

We also participate in ring tests and they are organised by reputable laboratories and that is because we have to check our results to see if they are comparable to those of the other participating laboratories and these we make frequently. So, Amorim has now moved almost entirely from sensory analysis to chemical analysis for quality control and other major cork producers in the area are doing the same. We are sure it has made a very significant difference to our ability to avoid TCA in the corks that we supply to the wine industry. Thank you.

SEFTON Thank you Stefan. We now have time set aside for questions and for discussion, if you just wait for the speakers to come forward. Miguel will sit by the control panel so that he can put talks back up if people want to refer to particular slides, and I would like to also welcome Ed Carr who is the Chief Sparkling Winemaker for Hardy Wines and he has also agreed to be on the panel, so thanks Ed. Can I ask these people to come and sit forward because it might be a little easier that way and can I also ask you to please introduce yourself and your affiliation when you are asking questions. And I believe that if our questions get too difficult, some of our speakers have assistants in the audience to help with some of the questions. OK it is open to you on the floor.

PANEL DISCUSSION

PAUL: Paul White (Australian Gourmet Traveller Wine)

WHITE The first question I have is for the second last slide. I am Paul White and I'm from Australian Gourmet Traveller Wine and my understanding is that the threshold for sensory perception of TCA is about two nanograms for white wine and about four for red wine. Is that generally understood? And what I saw from that last slide is that you statistically analysed, you're just at those two levels, that would imply to me that you would still smell the TCA with the chemical analysis or am I missing something in this…?

SEFTON Perhaps Stefan or Miguel would like to answer that?

WHITE It in the control chart… am I missing something in the control chart?

CABRAL Let me go back to the control chart. Look what the control chart is doing, what it is for: to control the daily run of our analysis. And we choose two control standards, one with 3ng/L, it is here, and another one with 6ng/L and these are the results that each day and each night we have just with these control standards. What for? To see if the values of that run are OK or not, if some problem happens with the machine. So the theoretical value is three because we prepare a solution of 3ng/L, and the results, the pink dots, correspond to each of the values obtained for that control, which theoretically should be three. So making a statistical analysis of all these values (and these values come from July last year until now), all the results together give an average, plus two standard deviations give this green line. And so, if you have any results of that control sample—not results of a normal sample, of their control standard—if you have one result outside of that green line, then that means that the precision of that machine on that day was not good and so we have to repeat all the runs of that day. So, it is a way of controlling the results that we have reached. Strict controls each day.

WHITE So, this is just basically for calibration of the machine?

CABRAL Exactly. Yes. It is quality control.

UNKNOWN He did say that it was really up to the complexity of the medium as to how people distinguish TCA. So he wasn't actually giving values.

SEFTON So, could we have another question please?

WATSON Bruce Watson, Constellation Wines, Pacific North West. Before we leave this slide, does this mean that we should be reporting these TCA values for a particular batch of corks, above plus or minus 1ppt? Is that the normal, the statistical range, like if we say this batch of corks is 1.3, it's really plus or minus about 1ppt on top of that?

CABRAL That is exactly it.

DAHL You may actually forget we are actually analysing a concentration, not at trace levels, they are sub, sub trace levels, very low. And we have validated all the methods vis-avis all the instruments, according to the ISO, there are eight or nine points to follow, to be really sure.

SEFTON Are there any other questions on this topic, before we move on to another topic. Some one up the back there…

LIMMER Alan Limmer from New Zealand, I'm not quite sure how far back you need to go but there was a chart with the sensory evaluation levels of TCA. The one with the water and the wine. Does that suggest that in a simple medium like water the nose is still more sensitive than the analytical technique?

DAHL Yes, plus, TCA doesn't like water. So the higher the ethanol level, the less is the release of the TCA. So that means, in water if you have TCA, even low levels, they will come up to the headspace much easier.

SEFTON Richard?

GIBSON Richard Gibson, Grape and Wine Research and Development Corporation. Related to this again, the analytical techniques that you use Stefan, the technique of salting out, is absolutely fascinating. I haven't struck it before. Can this be used in a sensory environment as well to decrease the sensory threshold of TCA in sensory by salting out?

DAHL I don't know. Because you are probably not only salting out the TCA selectively but you are salting out everything, then it is up to your nose if you can distinguish this amount of TCA better, or the complexity of compounds that you get in higher amounts. The mass spectrometer detector can and I can separate it in the column, but it must be a question of whether the nose can separate as well.

SEFTON I think the chap behind Richard had a question…

GODFREY Hi, my name is Sandy Godfrey, I'm a student at Adelaide Uni and I've got a question about the analysis technique actually. You said you choose 50 corks from each batch of 10,000 and you put them in one jar. How does that provide a representative sample of cork contamination?

DAHL We come across this question everyday. It is a good question. Because it is a very logical question. No you cannot. What we are doing is to take as many samples as we can…no, let me take it from the other side. If we get a positive sample from that batch it is reported and we take directly two or three more samples of the same batch, because everything is statistically done. You neither know if you have one cork of these 50 that has perhaps 100 ng/L and makes all of the value on its own or if you have 49 corks which are more or less (contaminated).

GODFREY Well, I guess the question is, if you get some sort of result, how do you go… what percentage…

DAHL Well, the study by ETS. That is why I referred to a tool. We have actually a tool to say whether to reject or accept a batch. And that tool is based on the study that ETS has made. How the cork variation is and what you can detect with 50 corks.

SEFTON Sorry, I would just like to ask Stefan a question on that, to follow up because I think that you are hitting on something here. Do you ever get false negatives by sampling large batches? Say you had 5 out of the 50, which is 10%, all had 3-4 nanograms of TCA in them, but that TCA was able to be absorbed by the 40 good corks. Do you think it is possible to get a false negative result? And have you seen examples of this, where you've tried soaking them individually or in a batch? And is that a danger with soaking large numbers in a batch?

DAHL No, we have found… You have a percentage of 'risk' corks when you have a value over 4 ng/L in the soak. So if the soak is over 4ng/L you normally have a risk factor that there are too many corks over the level.

WATSON I was going to ask a very closely related question which is, wouldn't there be a substantial advantage to soaking the 50 corks individually and then

pulling 50 results and combining them? Precisely for the absorption capacity?

DAHL That would be beautiful if I could do that, but it would take me more than my lifetime, because I have to analyse daily many batches.

SEFTON I think he is saying that you only do one analysis, you soak them separately, then combine them and just analyse that one liquid.

DAHL That is a study that we have actually done.

CABRAL Let me just add one point in here Mark. We are doing at this moment a significant number of samples. Why? Because we realise we have to start first from something, you know. And after ETS presents this study we realised that by sensory, which we did before, some corks from a batch of an enormous amount of corks, it's near nothing. So the corks were not even minimally controlled at that time. And so after ETS appears with this study, in February 2001 when we had our first machine, what we did was, we started to analyse in just one factory. We started to analyse to see if this methodology will help us in controlling our corks. And we realised that having this analysis per batch—so for instance if we have 50 bales per lot, so we have 50 results from that lot, because we have one analysis per bale—that allows us to see clearly…

[change tape]

CABRAL … lot, because the unique way to do it is analysing cork by cork, which is not possible in bales of 10,000, it's not possible. But, what Stefan was trying to show before… we are doing 8000 samples per month, and it is a huge logistical thing and we spent a lot of money on that because we realised that it is a big help in terms of separating the contaminated lots.

SEFTON A question from the back…

TYSON Paul Tyson from McWilliams Wines. More of a comment. I think it is encumbent upon all cork suppliers—we are talking about sampling here—that they record the statistical basis of their sampling, so as consumers of corks we understand the confidence levels that are implied in their sampling program. Because you just can't just sample practically every cork that you are going to use, so it has to be based on some statistical basis of sampling, not 'I think 50 corks will do', what are the implications of using 50 corks, so that you can say what is the confidence level and the AQL levels that you are going to be rejecting.

SEFTON I think that is a very important comment, the statistical basis for sampling is critical. I have to say, and I am amongst the large group of people who when presented with this sort of statistical information completely fail to understand it, and that is dangerous if someone gives you a lot of gobbledy gook, a lot of figures and lot of technical terms. I struggle with it and I suspect a lot of you might struggle with it too. I am not sure what the answer to this is but we need to have some confidence that the sampling on which these figures are based is valid for the set as a whole. Peter, you've looked at this a little bit, have you got anything to add? He is shrinking back into his seat… Peter has the same level of statistical expertise as I do, I am afraid.

GODDEN Peter Godden, Australian Wine Research Institute. I think it would be an excellent idea if the main cork producers standardised the reporting and the statistical basis as Paul suggested, I think that would be a great step forward. And I suggest that the companies such as yourselves would have a marked advantage if they did that, because of the quality and quantity of testing they actually do.

TYSON Just a point though, if they do base this on the 50 cork soak, if you do enough of those analyses you can extend that to give an indication of the performance of the corks, is that true? That via statistical methods that I don't understand, that if you've done enough of the soaking tests, that can be related to the performance of your cork?

CABRAL It can be related to the TCA contamination of that lot of cork.

SEFTON I have to say personally that I am not a fan of the big batch soaking, I think that it can be misleading, whether you soak corks in batches of 50 and do that many, many, many times whether that makes a difference. I would like to hear comments from cork users whether they are wine companies or cork suppliers, who have tried themselves soaking in batches, small batches of 4, 5, 10 whatever, and also tried soaking them one at a time. Have you got any experience of one providing more reliable results than the other?

TYSON I can only think of the one that we looked at and ultimately it has to be a one cork per bottle test, and I guess you have to take these things in stages. If you do your pre-screening where you are using multiple corks in the soak, your ultimate test is what you've got in the bottle. I think you can only build that up over time by, as a consumer of those corks, every opportunity that you get to look at your wine, you record the results that you get. So, over the course of the last 18 months we've noted almost 2000 individual bottles by cork supplier and grade and to me that is a very important factor in the selection of cork, the long-term performance of that cork.

SEFTON It is an after the event assessment of the cork?

TYSON Yes it is.

SEFTON Richard, could you tell us your experience with the corks?

GIBSON Yes, at Southcorp we focused mainly on five closures, five cork soaks. For a long time if we detected TCA in a five cork soak we would split them out and do individual soaks in fresh wine and that would invariably over the period that we evaluated that we invariably identified one closure as the source of the TCA. The odds were just so much in favour of one out of five, and so we went back to the five cork soaks, and we kept doing that for 6, 7, 8 months and the figures were just so dramatically in favour of one out of the five.

TYSON What number of corks were soaked in the batch? How many corks per thousand?

GIBSON A hundred. Twenty by five.

TYSON A hundred corks out of what sort of batch size?

GIBSON Variable batch sizes, nowhere near statistical level, but as a screening assessment.

SEFTON Are there any other comments?

GRIB Ben Grib, KWV South Africa. From the Californian study we know that the soaking method will represent the average of the individual samples and I have also seen that concluded by various studies that were done by cork suppliers themselves. So I don't think there is any discrepancies in that. This method in my opinion is very effective for process control in terms of detecting variation in your manufacturing process. I know that some cork suppliers do a lot of work in gathering statistics on individual corks behind these 50 cork soaks, in order to get the spread of the individual corks. As they know it could be one cork heavily tainted or it could be fifty corks slightly tainted. So I think the cork suppliers should hold up a statistical database of the individual corks over time. Because it is a lengthy process and they can't do it that often, but over time it should give us the confidence limits that we need. If we have enough data there should be enough proof of that.

SEFTON Can I just ask a general question. If you do a composite soak and you find there is a certain amount of TCA, how important is it for people here to know is it just one really bad cork out of the hundred that is causing the

problem, or do you have a one in five corks that are contaminated but at a lower level? Do they constitute very different problems for your organizations?

GRIB It's extremely important.

SEFTON So, you'd need to know the proportion of corks as well as the overall average of a batch of corks, is that correct?

GRIB We know that consumers on average detect 4 or 5 ng/L so if your average is 3 ng/L on all the corks, there is a good chance that the consumers won't detect it. But if you have 10% at 20 ng/L, they will definitely detect it and they will complain about it.

SEFTON So you really need to go beyond the multiple cork soakings if you want more information on that particular product.

UNKNOWN On top of that we are arguing about thresholds and that sort of thing, I mean, I would say that if you are looking at a batch of 50 corks and you get TCA from them, why would you use the batch at all? Why would you bottle your wine with that cork from that batch if it is going to contaminate your wine in any way?

SEFTON So, you are saying that if you had one nanogram…?

UNKNOWN You wouldn't know I guess…

SEFTON That's right, you could have ten corks with ten nanograms, which is a serious problem, or they could each have one nanogram which might be a… but you need to know don't you…

UNKNOWN At what point do we not use these corks for these wines?

SEFTON Other questions or comments?

MEZYNSKI Dave Mezynski, J. Lohr Winery in California. I am under the impression that the 50 corks that you are taking represent a fixed number per batch, I mean it's not variable from 10,000 to 100,000 or 200,00? Do you need a bigger number?

CABRAL It's 50 per bale of 10,000.

MEZYNSKI OK. There was a publication on military sampling and you're right. We get ours done with military sampling where you have to take so many corks out of a bale. I have a formula, but I couldn't really tell you how I

arrived at it. [inaudible] When you are really looking at corks and cork sampling, what can you do? I know there was a winery that soaked individually one per cent of all their corks. It was a small winery, but even with that being said, talking to the people, they said they liked to do it but all the time it pinched, they couldn't do it all the time. So, just doing one per cent I don't know that you are going to eliminate all TCA. It is just a way of simply looking at a batch to tell you what is really bad or what is really good.

CABRAL In my opinion, this system is exactly for that. It is a production control where we clearly know if a batch of contaminated corks is really bad or not. And for instance, if we have a batch of corks that have been contaminated by absorption of TCA from the environment for instance, it will be detectable by this system. So like I said, the individual corks are much more difficult to detect it with this system.

MEZYNSKI ETS ran it enough times to figure it is pretty consistent. They can soak 50 and then soak another 50 and they are getting the same numbers in a very consistent matter.

CABRAL Yes, that study is published by ETS and it's on the Internet, but probably it was one batch of corks.

SEFTON I think Richard and then Tony to make a comment…

GIBSON Richard Gibson, from GWRDC. The quality assurance techniques will only work if something happens to the batches that are rejected, if they are removed from the cork supply system. I did read that you were talking about treating those rejected batches with a TCA removal technique and I would just like to hear some comments from the cork suppliers about what happens to the batches that are rejected?

CABRAL So from our side what we do with the rejected batches is very simple. All lots of natural cork stoppers that have more than 4ng/L after punching, so an average of the bale by bale that Stefan explained, we pass them through ROSA at this stage. So, all positive batches we pass through ROSA. If after ROSA those batches still have some value above 4 ng/L, we repeat ROSA on that batch.

This is for cork stoppers. For cork granules we do exactly the same thing, except we only pass through ROSA lots that have TCA up to 20 ng/L. The other lots of granules go to flooring and insulation and things like that, because we don't use them to make cork stoppers.

GIBSON The natural cork then, that is ROSA treated potentially get tested twice and then passes…

CABRAL Just the positive lots…

GIBSON Yes, but is there any identification of those in your system, how can wine makers be sure that have not received the double ROSA treated corks?

CABRAL They cannot and it is not important to know that. All lots have the same specifications and in production all lots have the same specification. So if ROSA treated lots have the same specification of lots that are not ROSA treated, we mix them together.

SEFTON Tony first had a question I think and then the…

TELFER Tony Telfer, Managing Director, Amorim Cork Australia. Just following on from the discussion before about the limitation of quality control and GC/MS, perhaps a comment from Stefan or Miguel. If that is taken in the context of each step having its limitations but if you have a total program from the forest floor all the way through, each step combined reduces the risk overall.

CABRAL Tony, it's logical, that is the reason why I didn't just present ROSA as a miracle solution, because it is not. We have a group of preventive measures that allow us to have less and less contaminated batches of cork. And so we have ROSA to try to solve the problem in those batches. So that is the reason I made that first slide. I didn't want to go through all the details of what we do, but we try to prevent as much as we can. A simple thing like separating the lower part of the cork bark that is in contact with the soil. It is a small detail, but when we asked the factory to separate the lower part of the bark, we asked them randomly to send these parts and we analysed them and we see in some cases 500 ng/L. So, that amount of TCA, if we didn't separate that bit of bark, it in the boiling it will be cross-contaminating the others. So in all these preventive measures it's important to get a low a level as we can get.

SEFTON A comment or question from over here?

LIMMER Miguel, you mentioned in the ROSA treatment for the rejected corks on the evaluation test, you are putting whole cork stoppers through that test as well as granules?

CABRAL Yes. In my presentation I showed you results from the independent validation for granules but also the independent evaluations from Excell in France for cork discs and cork stoppers. But because we got deformation in those corks—because we are using heat, it's steam—we decided to go ahead with ROSA just for contaminated batches of cork stoppers and cork discs, not for all cork discs and cork stoppers in production as we do with

granules. Because the problems with granules after ROSA are not significant. So, after having one year of experience with this situation—positive lots pass through ROSA, non-positive lots don't pass through ROSA—then the board will have to decide if they want to pass everything through ROSA or not. All this is a question of equilibrium, of balance.

LIMMER With the whole cork in the ROSA system are you confident then that you are extracting all TCA? Given this was a failed batch are you removing all the TCA from inside the cork?

CABRAL No, from the experiments we have made from before, the TCA in the interior of the cork is not removed by ROSA. It is not easy to make that experiment because in order to analyse the TCA interior we destroy the cork, so it is difficult to see what was there after ROSA and before ROSA. However making analysis in a significant number of samples after ROSA and going into the interior of the corks, we have some corks still with TCA on the interior.

LIMMER Do you think in time that it will go into the wine?

CABRAL Well I think that Mark Sefton can answer that question.

SEFTON Well I will make a couple of comments. Firstly we haven't analysed nearly as many corks as these guys have, but we have analysed a few hundred and I would say that 90, 95 maybe more per cent of all the corks we have ground up and analysed have TCA in them. So, it's part of being a cork, having TCA in it. But most corks don't cause problems in wine, and that indicates that the TCA that is in the interior of the cork, unless your cork is going to sit in wine for a hundred years or more, isn't going to migrate into the wine.

Now, we have since done another experiment where we took a whole lot of bottled wines, some had just been freshly bottled so the corks were new, others were 10-20 years old so the corks were starting to get a little ropey around the edges, we spiked all of those bottles with 1000 nanograms—now that is a lot of TCA, 1000 nanograms—on the outside of the cork and we left that for 3.5 years. And after 3.5 years not one bottle had any trace of that TCA in the wine, and when we dissected the cork there was seldom less than 1% of what was added had got even to the half way mark of the cork. So, the migration of TCA is extremely slow.

LIMMER Was that just with natural cork or agglomerate corks?

SEFTON That included the agglomerate corks.

LIMMER So you're really saying that it is a superficial thing?

SEFTON Yes, that is what it appears to be. Now what I can't say is whether TCA can migrate through champagne discs because they are punched differently, you've got the disc here and the lenticels are parallel to the long axis of the cork, whereas with natural cork the all the lenticels are at right angles. So, a champagne disc may behave different, but I don't know. We don't have any evidence on that. But these involved natural corks, of all different bleaching types and they also included agglomerate corks. Agglomerate corks seem to hold onto the TCA a little bit better than the others, but the TCA didn't go through the corks any faster than with the natural corks.

WHITE Was that from the liquid from the outside, when you're actually polluting it with these high levels from the outside, or is it just painted on or something?

SEFTON We added it as a solution, we had this little drop of solution and you could see the cork soak it up and then we put some aluminium foil over the outside. Now, notwithstanding that, over 3.5 years more than half just disappeared into the atmosphere, but of what was left, which was up to 40% of what was added, it was nearly all on the top third of the cork. Hardly anything had gone through.

WHITE Having seen some corks when they may have a third of it soaked… a very old cork in wine that has been in contact for a long time, you can have a lot more liquid exchanging in the interior of the cork.

SEFTON Well, some the corks that we dissected were very old, that is they had been in the bottle a long time before we added the TCA and it didn't make any difference. I don't know why. We sort of thought maybe the old corks might behave differently. Just as we thought the agglomerates might behave differently, but they didn't, not in any significant way.

UNKNOWN Yeah Mark, just one thing I can't understand though. If the releasable TCA is in the external lenticels of the cork, and the internal lenticels actually, I've heard the theory that they actually trap the TCA there, that the TCA can't migrate through the cork, then in some of the results that we have seen after 12 or 18 months, there has been an increase in the TCA measured in the wine.

SEFTON Correct.

UNKNOWN So is that due to the wine penetration of the cork and then allowing it to transfer?

SEFTON What I said was that the TCA migrates very slowly through cork. Migration is not non-existent, so that means that presumably in the first few weeks when you put a cork into wine, TCA up to a certain depth—it might be a millimetre, we don't know—might be extracted. And then the TCA that is just below that in the cork is extracted more slowly. Now we don't know what these levels are but, ultimately if you leave the cork in wine long enough it will all come to equilibrium, but whether that takes 100 years or a 1000 years I have no idea, my research graphs don't run that long, which is probably just as well. It's not an all or nothing thing, you know, superficial TCA can be exchanged very rapidly, in a matter of a few hours. We know this from the soaking tests. But that doesn't mean that anything that isn't exchanged in a few hours can't be exchanged at all, it's just that the deeper into the cork it is, the more slowly it will be extracted out. And there comes a point, and I don't know whether that is 1mm or 2mm or what that is, where for all intents and purposes, the migration is so slow that it doesn't matter.

UNKNOWN Has anybody seriously looked at sites or forests where they might find different areas of forest regions where they have higher incidence of TCA and then isolating it? I have heard some correlation there but it's a little fuzzy.

CABRAL You are anticipating what I am going to say in the next presentation, so I am not going to answer your question.

SEFTON And if he doesn't answer your question then I will give you first question next time. One and then George and that will have to be it I am afraid.

WATSON A very brief question here. We still do two cork soaks where we do a whole bunch of bottles. We find that it gives us an overall character for the lot, which we think is more important for non-TCA taint, at least what we think is TCA taint, I am not so sure about that anymore. The questions is, how well linked to TCA concentration are these other compounds, which we suspect may be linked to cork problems… do we need a test for these other compounds? Which compounds? And when are we going to get it?

CABRAL So many questions and a very simple answer. I don't know. It is obvious that you have several different compounds. We have not just TCA, because cork is a material that absorbs a lot, like plastics for instance, plastics absorbs a lot too. So, we have not just TCA, but in general what is published in literature is that the main cause is TCA. But we have heard from Mark Sefton that he found a new compound, a very interesting compound that is probably justifies some of these other taints that we are not able to analyse. But to make a GC analysis, we have to make it for TCA and then we have to make it for the other compounds. On the other way and that goes directly to the question I want to ask Mark. If we use

methods to treat corks—granules, discs or corks stoppers—the question is to know if those methods are specific for TCA or are they mainly an open process that can clean the other compounds. And so the question is, do you think Mark that steam distillation is able to clean methoxypyrazine? It's an obvious question that I have to ask.

SEFTON Well, this particular methoxypyrazine is more volatile than TCA. It has a lower molecular weight, and although we haven't done any specific experiments with this particular compound, related methoxypyrazines are less soluble in cork. TCA is very soluble in cork and that is both a problem and a blessing. On the one that is why cork has TCA in it. On the other hand when the cork goes in the wine most of it stays in the cork, which is probably just as well. With this methoxypyrazine, yes if you've got it on the cork and you put it in the bottle, ultimately it is all going to go in the wine. But on the other hand because it is less strongly absorbed by the cork, or we think it will be, it should be possible to completely remove it by any of these processes that we have heard about, whether it be extraction or steam distillation. It is volatile, it is not highly soluble in cork. Before worrying about this, what we need to do, someone needs to do, is to screen wines and see how big a problem is this. I mean because every man and his dog have screened wines now for TCA, whenever we get samples for cork taint for example, if we are asked to analyse them, we analyse them and most of the time we find TCA. Occasionally we get a wine which is pretty musty and it's got 1 nanogram of TCA, and you think now is our panel particularly sensitive today, or are we missing something.

So, yes there could be and almost certainly are other compounds that contribute to cork taint. How important they are in comparison to TCA is difficult to say. There is not even an agreement on what percentage of wines on the market place are affected by TCA. I mean, if you ask a cork supplier it is a small percentage, and if you ask a wine writer it is a very high percentage and you will get all sorts of figures in-between. The consumer is the one you really need to ask. So, those are the sorts of questions that need to be answered first. But I think that notwithstanding that the methoxypyrazine should be amenable to steam distillation or CO_2 extraction. And if they don't work I can tell you that sulphuric acid will certainly take care of it. I'm not so confident of the structural integrity of the cork afterwards.

Now the next session is based on cork as a barrier to oxygen and we have another 30 minute discussion session set aside after that. So, there are opportunities if questions on permeation become a bit thin to revisit some of these topics. First I think we can have a short break, then we have two more speakers and then a final discussion. So if you could be back here in ten minutes please for the next session.

SEFTON Our next presentation is by Miguel Cabral and he will be talking on the cork's role as a wine seal. Miguel over to you.

CABRAL What I'm going to present now is a brief overview of the other areas of research that we are doing now at Amorim and of course in cooperation with others. Probably you are waiting for lots of results, but I am not going to present lots of results. I am just going to tell you what are the areas of research and then Jean-Marie Aracil is going to present results that he has in one of those areas. So, the current areas of research, still in TCA, oxygen permeability and new glues, are basically what we are doing now.

Concerning TCA and once we got ROSA, so once we got a curative process, we wanted to know a little bit more about the origin of TCA, so that goes straight to the question we were asked in the discussion. So, we study the origin of TCA in order to see if we can get preventive measures at that level which can actually reduce as much as possible TCA before the cork arrives in at the factory. And so, we started this work based on results from Juan-Jose Rubio Coque, who is a Spanish researcher who has produced very interesting results. He clearly proved that 2,4,6 TCP, tri-chlorophenol, is undoubtedly the main precursor of TCA in cork.

So how did he do that? He went to the forest to isolate the several different micro-organisms in cork. We know that cork is in the forest for nine years, growing in the tree, and then we have also a period of stabilisation (after harvest) of around six months minimum. And during all these periods we can have micro-organisms grow. And so he was trying to isolate that fungus, several different fungi, and then he tried to produce TCA in sterilised pieces of cork with that fungus in the presence of precursors. Because as you know, TCP and the other compounds, but with TCP mainly, in the presence of fungus is going to produce TCA. So, he realised that the amount of TCA produced from TCP in these pieces of cork, which he sterilised first and then contaminated individually with each mould, he realised that the amount of TCA produced by each mould is different.

As you see here, the bio-conversion of TCP into TCA from this group of fungi was very low, which he called non-producers of TCA. Another group of moulds here, which are low-level producers, this is the percentage of bio-conversion from TCP into TCA. Another group of medium producers. as he called it, the percentage of conversion is higher. But then a group of high-level producers, where we have *Trichoderma viride*, *Fusarium oxysporum* and *Trichoderma longibrachiatum*, the moulds which have higher conversion of TCP into TCA. So what this means is that we have, or we can have, in the environment fungi that are able to transform TCP into TCA, and in this case in cork. And we were

able to isolate this fungus from cork. So what, Juan-Jose Rubio Coque did was… he was trying to see if *Trichoderma longibrachiatum,* the mould that gave higher production of TCA, was able to produce TCA, not just from TCP but from other sources like different DCP, hexachlor-hexane, pentachlorophenol, penta-chloroanisole and so on. But look, in the presence of or absence of sodium hypochlorite no doubt the great amount of TCA was produced in the presence of this precursor, 2,4,6 TCP. And so we get a bio-conversion of 34.72%. So this clearly indicates to us that TCP is the main precursor of TCA in cork.

So, what we are trying to do is to see what is going on in TCP in forests. And for that I am going to show some results we have. We went to forest in Portugal, in Spain and also in Morocco and we have some samples from Tunisia and Algeria, and we will have from Italy from Sardinia. And so we went to the forest in order to analyse, from samples from the piles, we wanted to see the amount of TCP and TCA already there. Not just TCP and TCA but other precursors—PCPs, all the other phenols and anisoles and so on—but they were not actually significant at all, they were not there. So we fix just on TCP, tri-chlorophenol and tri-chloroanisole.

So, we got random samples of thin and thick cork, and for TCA samples we got them from the edges and also the interiors of cork piles, and the results that are here. These are not final results, because the number of samples is very different. But you can see that we have much more samples from Portugal, because it was very easy to get, some samples from Spain, some samples from Morocco, and you can see that the amount of TCA is not very high in Spain. We were not able to get one sample with TCA. This is the percentage of TCA as you can see between 5 and 10 ng/L or more than 10ng/L and these colors represent less than five.
So the great amount of TCA is not really there with the exception of Morocco, where we have already some positive samples. But when we talk about TCP we have some potentiality here. We have some already TCP in the forest, mainly in Morocco, but from that we cannot extrapolate. We can't say it is higher in Morocco, because the number of samples is not at all comparable, so we cannot say that.

So the point is that TCA is a problem but TCP is where we have to be focused if we want to beat the problem at the source. And so, for that we have two approaches and one we are interested in, and the approach is this. We know that there are fungi that are able to degrade TCP without producing TCA and that fungi, and we are going to try to find that fungus in the forest, in the soil, water, earth, cork, and then once we have that fungus, we try to spread it out in forest just to see the result. This is one approach to the problem.

The second approach is to try to produce genetically modified fungus with this capacity, so degrading TCP without producing TCA, and do the same. So this project it is not yet approved, but we applied for a grant to the European Union, in conjunction with a university in Portugal and a university in Italy, and we are waiting for the answer, and then we are going to see the result. I have to tell you that the idea of the project is interesting but I am a bit suspicious about results that we can have. Because in a serious field we can spread some micro-organisms in order to lead something into one direction, but that is during six months and even that is difficult. With cork we are talking about nine years in the forest, so it is much more difficult. However, we have to see what is going on.

So this is one interesting area of research that we are trying to do. The second area of research is, can cork stoppers allow micro-oxidation of the wine? I say question mark because this is the subject that everyone is talking about, random oxidation and so on. After the work of AWRI, Peter Godden, a paper came out in 2001—and already Mark Sefton has showed some results of this and Jean-Marie Aracil has too—that showed screw caps gave high reductive characters after 18 months. That was the result from Peter Godden. So this indicates to us that the cork enables some micro-oxidation—or not, I don't know. It is a question mark, so we are trying to do some work on it, just to see what is going on.

So we are doing that in collaboration with the Faculty of Engineering at the University of Porto. And there have been two different approaches. One has been just with bottlenecks and corks inside, so without wine. The second one we are doing in France in the University of Bordeaux, we are paying a PhD student there, to do this work with wine. First of all we want to determine the best measure of permeability and we will choose between these two methods—time lag and Wicke-Kallenbach. Don't ask me too much about these methods, the University of Porto know it, I don't want to know too much about it. And once we choose between these two methods, we are going to do exactly what you showed in your last slide Mark. It is to see if there is some permeation, where is it from. If it is from the interior, from the middle of the cork, if it is from the cork or the glass, or even if it is from after squeezing the cork into the bottle.

So, these are questions that we are going to try and answer with this project, which is in its first three months. The bottling experiments that are happening in the University of Bordeaux are unfortunately not going so well, I guess. But, we are using some very different types of closures, one synthetic and several different types of cork closures, and the idea was to try to make a big variation in storage conditions and see the effects in terms of oxidation in wines. But unfortunately I don't know if we are going to get something from this.

Finally, the glue research. We do not do research in glues at all. So, we ask the glue producers, the big chemical companies, to develop this kind of glue for us. And why are we asking that? Well, the glues we use are polyurethane and casein, which is still used in the Champagne region, to glue the cork disc to corks. So the idea is to try to use different glues, and there are some candidates with very good results. Aliphatic iso-cyanates, ethylene vinyl acetate for discs, we are getting very good results from this, and latex glues. I am not showing you any results of these because I don't have final results. However, with one of these glues, we are already doing some industrial trials. Let's wait to see the results.

So, these in general are our main areas of research at the moment. We are tyring to see what we can get from this, and the idea of presenting this for you is just to tell you that once we start, four years and a half ago with this research and development program, we don't want to stop and we will continue. Thank you.

SEFTON Can I ask the panelists to come to the front again and we will talk. According to my watch we have absolutely no minutes left for discussion but I am sure we can fit some time in. Peter did you want to make a comment?

GODDEN Peter Godden, AWRI. Miguel, with the work you doing with different glues, what problems do you perceive with current glues?

CABRAL Basically we now use polyurethane as you know, putting aside casein, and it is probably a little bit dangerous not to have an alternative. That's basically the reason why we try to get other alternatives. That's the reason I put there, 'cheaper glues'. We don't really have problems with these current glues. All the studies that have been done with technical corks, agglomerate corks or Twin Top corks didn't show any glue odour or anything like that. The glues are FDA so we have no problems with our glues. But it is a little bit dangerous to have just one alternative, so that is the main reason why that we are trying to…

GODDEN Have you looked at the possibility that there could be a glue that could act as a barrier to TCA?

CABRAL Well, that is not our idea. However, it is not finished yet but we are trying to do a deal with a university in the UK, where we are… We don't want to say to them what we have, we want to see what they can give us. And that direction is one part of the point. But we are not really thinking in that direction right now.

UNKNOWN When you are measuring the oxygen permeability, do you use the same bottle every time or do you use different bottles?

ARACIL No, we don't use bottles, we use cells. The cells are in aluminium and for each measurement we use one specific cell, but with the same dimension and in the same condition.

CALOGHIRIS Martin Caloghiris, Vinpac. I had a question for Jean-Marie. Can you confirm the effect of vacuum under the closure in relation to permeability?

ARACIL It is very difficult to confirm exactly the effect in the real system on the bottle line. It is very difficult to see exactly the effect. But we have made a lot of measures in this device and when we change the pressure difference actually, we change the speed of the transfer and the quantity of oxygen we transfer through the cork. And we have built some mathematical models and we don't have for the moment the most important things—the speed of consumption of oxygen by the wine itself, the variation depending exactly on the storage conditions and the type and the composition of the wine. And we have made some studies on this level to have, not all the information … it's not possible to have all the information. The parameters change with each type of wine, but some guidelines

SEFTON Richard I think you have a question?

GIBSON Yes, just to Jean-Marie again. In the cell Jean-Marie, the closure section is not compressed?

ARACIL Exactly, we struggle with ready-to-use cork stopper. One very important thing is the compression ratio if the glass manufacturer changes the size, not very big but small, it changes with a little impact the speed of the transfer. With this type of closure, I never spoke about the transfer on the interface between cork and glass. Depending on the mechanical properties of the cork, this transfer is very, very, important. It is more and more the ratio between transfer through the stopper and on the interface can change a lot depending on the condition and type of product.

SEFTON Other questions?

UNKNOWN Those fungi that potentially generates TCA, to me one of the questions is are there fungi out there they will also consume TCA? Is that a net effect? Was there any evidence in that?

CABRAL As far as I know, no, there is no evidence of that. What there is evidence of is that actually they can consume TCP, tri-chlorophenol. And do you

know why, because TCP is toxic to them and so once TCP is not in a high concentration they change it in order not to die. It is a survival system.

SKOUROUMOUNIS We've heard about TCA and how Amorim and the other companies are trying to get rid of TCA in the manufacturing process. But one thing we haven't addressed is the environment, because cork is actually a good absorbent of TCA in the environment, and although you may go through these beautiful processes of cleaning up the cork, it is the environment that can also be detrimental where the cork can act as a sponge. It may not be the cleaning of the cork that's the problem, it may also be the environment. I have been to a lot of places, I won't say where, into a car park where you can smell TCA in the environment. Does anyone have any idea where we can prevent the surroundings—say you have 10,000 or a million corks in a shed—is there any way that we can have a protective barrier for the corks from the environment, depending on where the corks are stored?

CABRAL The environment can be a big problem and so the curative measures that we show today are one of the measures that we are using to reduce that absorption of TCA that actually happens from the environment. You know there are several different ways to try to achieve that. One of these, which is probably not easy to do, is to keep corks with as low humidity as possible and secondly keeping them in areas where the air has to be filtered, and that way we prevent the entry of air contaminated with TCA. But we have… for instance with TBA. We have not talked today about TBA, tri-bromoanisole. It is a new fashion that everyone is talking about, Pascal Chatonnet has shown the origin is a fire retardant. So, we have that problem for the same reasons, the environment.

SEFTON I think that what George says is very correct and that is that cork is a very absorbent material and it's not just TCA in the environment that can be absorbed, but other contaminants. This is particularly an issue for Australian consumers. If the cork is produced in Portugal they go into a container for six months, so, the cleanliness of that container is essential. And then they may be stored for a period of time before use. But this is an important thing that users have to be aware of and even if corks are clean when they leave the factory it' s still worth checking them before bottling, just to make sure that something unfortunate hasn't happened in the meantime. It is twenty past twelve. What time do we have to be out of here? Does anyone have any other burning questions… if not then please help me in thanking the speakers.

END OF SESSION